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THE CYTOLOGICAL BASIS OF MUTATIONS[1]

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IT is nearly three decades since the publication of the mutation theory by de Vries, and during the intervening years the term mutation has been redefined many times in the light of increased knowledge. The processes in Oenothera on which de Vries based his conceptions have since been analyzed into a variety of different types of germinal change. In the meantime, the genetical work with Drosophila and other organisms as well as Oenothera has raised a great superstructure of cytogenetic fact and given us fresh insight into the architecture of the germ-plasm and its variations. It can now be recognized that a mutation is a change *of any kind* in the germinal material. Having occurred in the germ-plasm, it will be transmitted by mitotic division and inherited, provided only that the particular germ-cells in which it occurs, or their descendants, survive to take part in the production of a new individual. I shall return again later to this definition of a mutation, as some geneticists would probably not be inclined to agree with it.

During the first decade of cytological work with Oenothera, the counting of chromosomes made it possible to classify the mutations into four types: (1) the $(2n+1)$ forms, now called trisomics, (2) tetraploids, (3) triploids, (4) 2n forms in which no change in chromosome number was involved. Of the last group, a few were recognized as simple Mendelian or gene differences,

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such as *brevistylis, nanella* and the red factor of *rubricalyx.* The remainder, of which *rubrinervis* may be regarded as the type, were not inherited in such simple fashion, and their origin was regarded as more obscure. In this first analysis (Gates, 1915) of Oenothera mutations, it was naturally assumed that there would be seven $(2n + 1)$ forms or primary trisomics, corresponding to the seven pairs of chromosomes. But since the study of chromosome linkage has led to the recognition that the chromosomes of a linked group are not in pairs of strict homologues, the number of possible trisomics arising from certain forms has become greater. Thus in *Oe. Lamarckiana* with a ring of 12 chromosomes and a single free pair, we may expect, as pointed out by Håkansson (1930), 13 different primary trisomic mutations, provided that all are viable; and a species such as *Oe. muricata, Oe. eriensis* or *Oe. novae-scotiae,* with its 14 chromosomes linked in a ring, might be expected to produce 14 more or less clearly distinguishable trisomic types. But although, as shown particularly by the work of Sheffield (1929), non-disjunction is of relatively high frequency, at least in the pollen meiosis, yet records of trisomic mutants from such wild species in cultivation are as yet remarkably few. It appears that pollen grains with 8 chromosomes are either non-functional in the great majority of cases or lose their extra chromosome in the nuclear divisions of the male gametophyte. These facts detract from the evolutionary importance of non-disjunction in the genus Oenothera; and since all known wild species of Oenothera have 14 chromosomes, we may assume that any forms which may have appeared as a result of this or other chromosome aberrations have been unable to run the gauntlet of natural selection and achieve permanency.

All modern cytogenetic studies emphasize the remarkable constancy of the germ-plasm, although this appears to be greater in some groups than in others. The chromosome linkage maps of Drosophila, for example, show that as regards *D. melanogaster* and *D. simulans,* the

genes occupy corresponding positions in each chromosome, except that a portion of chromosome III appears to have been reversed (see Sturtevant, 1929). Similarly in these species the evidence shows that in their whole history no genes have shifted from one chromosome to another of a different pair. In other words, there has been no translocation or crossing-over between non-homologous chromosomes since these species originated, because all the genes known occur in homologous chromosomes in the two species. It is found, however, that in *D. melanogaster* inversions of sections of chromosomes II and III are not uncommon. But they do not result in phenotypic difference or in cross-sterility.

D. melanogaster and *D. simulans* are so nearly alike that they were not recognized as distinct until 1919, and, although they will cross, the offspring are sterile. The two species show a high amount of parallel mutation, 27 of the loci that have mutated in *simulans* having also mutated in *melanogaster*. High mutability of a particular locus in one species is also correlated with high mutability of that locus in other species. Yet notwithstanding the remarkable similarity in the structure of the germ-plasm of these two species, there is a striking difference in their sex chromosomes. In *D. simulans* the Y is a straight rod about two thirds the length of the X, while in *D. melanogaster* the Y is J-shaped and longer than the X. This difference probably accounts for the interspecific sterility, and it shows that while the autosomes of these species have been undergoing a high percentage of parallel mutations their Y-chromosomes have become physically differentiated in structure through some process of which we have no present knowledge. The shortening of the Y in *simulans* and its change to a rod shape suggests that the short arm of the *melanogaster* Y has been lost, the spindle fiber attachment thus becoming terminal. This and similar evidence from Crepis and other organisms makes it clear that the different chromosomes of a species may undergo independent phylogenetic change, one chromosome remain-

ing stationary except for invisible gene changes while another is undergoing important structural alterations.

It is necessary to conclude that for millennia the reproductive processes of these species of Drosophila have been taking place with countless millions of mitotic figures in which the same spatial relationships of the germinal elements within the chromosomes have been maintained. For greater constancy or slower rates of change one must go to inorganic geological processes such as the weathering of rock and denudation of land surfaces. In the chromosomes we have some of the most complicated and labile of chemical substances, yet all the evidence shows that the structural relationships of the hereditary elements within the chromosomes are remarkably maintained.

It is now well known, however, from the work of Muller and others, that changes in the linkage maps of Drosophila are very frequent after exposure to X-rays. This but emphasizes the normal constancy of gene arrangement. The chromatin rearrangements so induced (Muller, 1930*a*) following chromosome breakage include (1) translocation, where one of the fragments becomes attached to a different chromosome, (2) inversion of a portion of a chromosome, and (3) deletion or loss of a chromosome fragment. From inversion by crossing over may arise (4) duplication of a portion of one chromosome. All these different types of displacements in Drosophila chromosomes have been analyzed by Muller, through the changes in crossing-over involved. It is important to note that while of common occurrence in the offspring of X-rayed animals they rarely occur in untreated material.

Although the effects of radiation are said to be most marked on rapidly dividing tissues, yet it appears probable that the compact gel state of the chromosome is less susceptible to the effects of bombardment than the loose and uncompacted state of the so-called resting nucleus. The extensive investigations of Goodspeed on the germinal effects of X-rays and radium in Nicotiana show

that the effects on the chromosomes are similar to those in animals. Goodspeed (1930) (see Goodspeed and Avery, 1930) describes resulting fragmentation of chromosomes, with duplication or translocation by addition of fragments to other chromosomes. Such fragments may be attached by a thread to another chromosome, thus resembling in some respects a satellite. The offspring of treated plants also contain trisomic and monosomic individuals evidently resulting from non-disjunction or non-conjunction.

It is well known from the work of Hegner (1909) and others that in Chrysomelid beetles the germ-cells are set apart at a very early stage of development, during the formation of the blastoderm, and they do not begin to multiply until the gonad walls have actually been formed to receive them. Thus the Keimbahn or germ track is set apart from the soma as a separate line of cell descent almost from the beginning of development. The same is probably true, though perhaps to a less marked extent, in insects generally. The contrast with conditions in flowering plants is striking. Here we find no distinction between germ-cells and soma until we actually reach the archesporial cells which are to produce the pollen mother cells or megaspore mother cells. The Keimbahn of a flowering plant is thus vastly longer, as regards the number of cell generations intervening between the fertilized egg and the definitive germ-cells, in higher plants than in insects. Conceivably this difference in the length of the Keimbahn may be correlated with a difference in the stability of the germ-plasm in the two groups, the fossil and other evidence suggesting that species are "longer-lived" in insects than in Angiosperms.

The work on the experimental production of mutations has been especially valuable, by providing abundant mutations for analysis, in leading to a further understanding of the various kinds of mutational change which can take place. The tendency of the Morgan school, however, has been to restrict the term mutation to a single

type of change, *viz.*, gene mutations. Muller (1930*b*), for example, speaks of the "spurious mutants" of Oenothera. In point of fact, the first mutation discovered by de Vries was *lata,* which we now know to be a trisomic. The history of this restriction of the term has been simple. Each type of mutation which has been cytologically analyzed, so that the nature of the process which produced it could be more or less understood, has been promptly removed from the category of "true" mutations. This has been done with the trisomics, the tetraploids, triploids and now, theoretically at least, with the mutations involving changes in the linkages between chromosomes, which are believed to be due to some form of translocation. All these have been excommunicated, leaving only the gene mutations, regarding whose origin we know nothing except that they can be produced with greater frequency by the action of X-rays. To be consistent, when we know something of the nature of a gene change these also will cease to be "true" mutations. There will then be no mutations left!

Indeed, as is well known, Lotsy has advocated the view that gene mutations, since they occur in organisms whose germ-plasm is not completely homozygous, are themselves a result of hybridity. This search for germinally pure organisms is a species of Snark-hunting which a number of investigators have pursued for two decades, but it is perhaps now generally recognized that such a condition exists chiefly in the mind of the biologist and that very few truly homozygous organisms are to be found in nature. Rather must we analyze the various types of germinal change which can take place in the evolution of material which is usually germinally heterogeneous to begin with.

To me it appears therefore more consistent to recognize all these hereditary changes in the germ-plasm as mutations of different categories, due to different kinds of germinal change and having different kinds of evolutionary significance. Probably no one will now deny the fundamental importance of tetraploidy and other grades

of polyploidy in the phylogeny of flowering plants. The tetraploid *Oenothera gigas* was one of the early mutations discovered by de Vries, but when we learned something of the way in which it was produced it was relegated to the category of "chromosome aberrations." A large number of plant genera are now known in which the tetraploid and hexaploid species are just as normal and frequently just as successful in nature as the diploid. They must have arisen at some time in one of the ways in which we know that polyploids do arise. The changes involved are germinal, and it appears to me that there is no shadow of justification for excluding them from the category of mutations, especially as they have so clearly played a fundamental rôle in the evolution of many genera. Indeed, in Angiosperms according to present knowledge polyploidy is the rule and its absence the exception. These are some of the reasons why I think the term mutation should be used in the generic sense, including various classes or categories of mutational change. It is especially among students of animal genetics, where polyploidy is of minor phylogenetic importance, that the term mutation has been so unjustifiably restricted to gene mutations.

I should like now to return to chromosome linkage, which has been the subject of so much investigation and speculation in recent years. The study of intra-chromosomal or gene linkage was largely developed before linkage between chromosomes began to be studied. Gene linkage is sometimes referred to as chromosome linkage, and as it is becoming increasingly difficult to avoid ambiguity and confusion in the use of these terms I propose the word *catenation* for the physical linkage between chromosomes, causing them to remain attached in chains or rings during the essential stages of meiosis, *i.e.*, in diakinesis and usually until separation takes place in the heterotypic anaphase. The evidence, so far as it exists (Gates and Sheffield, 1929*a*), indicates that in somatic mitosis the chromosomes are not paired according to their linkages during meiosis. Thus in *Oe. rubricalyx,*

which has four free pairs and a ring of six in meiosis, there is no indication of four corresponding pairs in the somatic metaphase.

Catenation was first discovered in *Oenothera rubrinervis* (Gates, 1908), but the number of chromosomes in a chain was not then recognized as fixed. Cleland (1922) afterwards found in *Oe. franciscana* a ring of four chromosomes in diakinesis, although it broke up into pairs on the heterotypic spindle. Later (1924) in *Oe. franciscana sulfurea* he found a ring of 12 chromosomes and one free pair, and this time the connections between the chromosomes persisted until metaphase. Since then, a number of workers have determined the catenation or fixed linkages in many species, mutations and hybrids of Oenothera. It is now generally recognized that each form has its characteristic catenation, which is usually remarkably fixed and constant in successive generations, and that these linkages account for the genetic linkage which is an equally striking feature of Oenothera. The chromosome connections limit the freedom of distribution of chromosomes which can take place in the reduction division; but in order that this shall be effective in determining that certain chromosome combinations shall always enter the same daughter nucleus the chromosomes must also have fixed places with relation to each other in the ring or chain. Since the ring is usually arranged in zigzag form on the spindle, alternate chromosomes normally pass to the same pole and therefore belong to the same complex, adjacent chromosomes belonging to different complexes. It therefore appears that in catenated species each chromosome in the ring or chain occupies a fixed position in relation to its neighbors.

While catenation is more characteristic of Oenothera than of any other known genus, yet similar conditions have now been observed in other plants, and it is possible that the process has the same meaning in all. A case which appears to be of a similar kind has been recorded in the Copepod, *Diaptomus castor,* where a ring of 3 double chromosomes is figured in the heterotypic meta-

phase, the other 14 meiotic chromosome bivalents being separate (Matschek, 1910). If this case is confirmed it would dispose of Muller's (1930*b*) argument that chromosome linkage (catenation) can not be expected to take place in bisexual organisms.

A considerable number of plants are now known to form rings or chains of associated chromosomes during meiosis. Håkansson (1925) found a ring of four in the diakinesis stage of one pollen mother cell of *Godetia amoena;* Belling and Blakeslee (1926) described chains of 3–5 chromosomes in trisomic Daturas; Belling (1927) figured the 12 chromosomes of *Rhoeo discolor* united into a ring; Kihara (1927) described rings or chains of 4 or 6 chromosomes in *Rumex acetosella;* Stow (1927), tetrapartite and hexapartite rings and chains in *Solanum tuberosum* as well as variously composed rings in Tradescantia. Darlington (1929*b*) has made an analysis of ring formation in Tradescantia and other Commelinaceae, while Meurman (1929) described rings of 4, 6, 8 and 10 chromosomes in the tetraploid *Aucuba japonica* with 32 chromosomes. Håkansson (1929), from an examination of Hammarlund's material of Pisum showing unusual genetic linkage, found that in certain plants a ring of 4 chromosomes was present, although most plants had seven free pairs. Richardson (1929) has also reported a ring of 4 chromosomes in a strain of Pisum from Tibet, and Gairdner and Darlington (1930) a ring or chain of 4 in a certain strain of *Campanula persicifolia.*

Belling (1927), in an important paper, gave a clue to the nature of these attachments. On the basis of specific attractions between chromosomes, he concluded that similar chromosome ends remain attached to each other and that chains or rings arise through interchange of segments between non-homologous chromosomes. More recently it has been shown (Blakeslee and Belling, 1926) that the so-called B strains of *Datura stramonium* when used in crosses induce the formation of a ring of 4 chromosomes in other strains. The remarkable results obtained by identifying the extra chromosome in the

various (2n + 1) forms (primaries, secondaries and tertiaries) and their crossing with B strains are of great importance in the history of genetics.

It is necessary to point out, however, that there are characteristic differences between the phenomena of catenation in Datura and Oenothera, and it is unfortunate that no detailed study of the method of synapsis in Datura has yet been made. Datura has the advantage that its chromosomes can be separated into different morphological types, while in Oenothera this has not hitherto been possible, although Levitsky (1929) has recently reported in *Oe. Lamarckiana* one pair of somatic chromosomes with appendages. This may correspond to the single free pair found at meiosis in this species. The other somatic chromosomes, he finds, fall into three groups: (1) three pairs with equal arms, (2) two pairs with unequal arms, (3) a smaller pair with unequal arms. Detailed study of the somatic chromosomes may thus yield a method of identifying homologous chromosomes in different Oenothera species.

In *Aucuba japonica* the 8 chromosomes of the haploid set are all more or less clearly recognizable by their morphological peculiarities (Meurman, 1929), so that the various chromosomes in the rings, and their position in relation to each other, can be recognized. It appears that when similar chromosomes are consecutive in the ring they usually pass to the same pole, and the very high frequency of 25 per cent. of non-disjunction is found in this tetraploid species. Lateral chiasmas, as described by Darlington, are also found, and interpreted as resulting from the inversion of a segment of a chromosome.

Of these various cases, Tradescantia as analyzed by Darlington (1929*b*) shows the greatest variety of conditions, including polyploidy, fragmentation of chromosomes, inversion and reduplication of chromosome segments, translocation and interchange of segments between non-homologous chromosomes, as well as the terminalization of chiasmata according to Darlington's hypothesis. This abundance of interchanges between chromosome

segments and fragments in the tetraploid *T. virginiana* is, as Darlington points out, accumulated and made possible by the fact that the species relies upon vegetative methods of reproduction. Under these conditions, the individuality of the chromosomes during meiosis is largely destroyed, and this is presumably why the species must rely upon vegetative reproduction for its continuance. It would be quite misleading to suppose that normal species with sexual reproduction could show an equal disregard of their chromosome individuality during the meiotic processes. Darlington's hypothesis thus assumes an amount of segmental interchange which is greatly in excess of that actually found in Oenothera, where each species, hybrid and mutant, has as a rule its fixed linkage which is rarely departed from.

Rhoeo discolor (Belling, 1927; Darlington, 1929; Kato, 1930) is the only form except Oenothera in which complete catenation has yet been found. These phenomena in Oenothera have therefore appeared in some respects unique. Before the persistence of the connection between chromosomes was recognized, the position they took up on the heterotypic spindle was attributed by me to lack of attraction between homologues. This view of course still holds, and more recently Cleland (1928) has also spoken of "disharmony" between the chromosomes, those which form pairs being regarded as relatively homozygous and those which fail to pair as heterozygous or carrying a number of different factors. I have been impressed with the fact that all the small-flowered species except *Oe. ammophila* have a complete ring of 14 linked chromosomes at diakinesis and heterotypic metaphase. It appears to be generally agreed (Gates, 1915; Boedijn, 1924; Broekens, 1925) that in the phylogeny of the genus Oenothera the smaller-flowered species were developed from large-flowered ancestors. The latter are found in South America and northwards into the Southern states, and we may suppose that the genus moved northwards in North America following the retreat of the ice. It was therefore suggested (Gates, 1928) that the northward

movement had been accompanied by crossing between species thus brought into touch with each other, this intercrossing leading ultimately to complete catenation of the chromosomes, since homologous chromosomes were so unlike in the hybrids that they failed to pair. Such species will breed true so long as the chromosomes occupy fixed places in the ring and so long as the homozygous recombinations are non-viable. This latter stipulation made it necessary to assume that in the hybrid species balanced lethal factors were present or that a rearrangement of certain chromosomes had taken place; otherwise they would be expected to segregate again into the parental types. Renner (1917) had assumed that in *Oe. Lamarckiana,* for example, the *gaudens* and *velans* complexes were no longer capable of producing homozygous viable types because they had been altered through exchange of factors. He also (1921) assumed that those complexes differed as regards a single pair of their chromosomes, a view which has since been given up. The view now generally adopted is that the chromosomes composing a ring are all genetically unlike, while the chromosomes which form pairs may differ in one or more genes but are nevertheless homologous in their basic structure.

While there are therefore certain difficulties with the hypothesis of catenation arising through crossing of species, yet it is not at present wholly excluded. But it probably requires to be combined with the theory involving interchange of segments between certain chromosomes in the process of meiosis in the F_1 hybrids. It can be definitely tested by determining whether crosses between species with partially catenated or wholly paired chromosomes can give rise to relatively constant forms with more or less complete catenation. No such case has yet been recorded, but crosses are now being made to test this point.

A fact frequently lost sight of in discussions of chromosome linkage (catenation) is that in all accounts of

meiosis in Oenothera except one or two recent ones (*e.g.*, Weier, 1930) the heavy spireme or pachynema has been recognized as a *continuous* thread without free ends, *i.e.*, as forming a closed ring from which the ring of 14 chromosomes end-to-end which is known to occur in several species is formed by constriction between the chromosomes. In some species the pachynema not only constricts at intervals to form the 14 chromosomes, but one or more free pairs of chromosomes are completely cut off by the severing of their connections with the spireme, while the remainder form a closed ring. In *Oe. rubricalyx* the four free pairs appear to be cut off simultaneously from the spireme, while in *Oe. ammophila* × (*biennis* × *rubricalyx*) there is some evidence (Gates and Sheffield, 1929*b*) that the three pairs may be cut off successively. If this continuous pachynema is a fact in all Oenothera forms, regardless of their catenation, then in this genus the chromosomes pass through a stage in which they are *all* arranged end-to-end in a closed ring. As the pachynema constricts to form the chromosomes, one or more pairs are cut off (the members of each pair being arranged tandem) while the rest of the chromosomes remain attached end-to-end because the spireme has failed to complete the constrictions between successive chromosomes. Thus the linkage of chromosomes which occurs in catenated species may be looked upon as a failure of the pachynema to complete its segmentation. It is possible that the linkages could be accounted for by a tendency for the constrictions of the pachynema to take place between similar genes. The continuity of the pachynema is thus a fact of fundamental significance in all interpretations of Oenothera cytology. It has been denied, we think on inadequate evidence, by Weier (1930). But the evidence on this point, from the work of Gates, Cleland, Sheffield, Håkansson, Oehlkers (1926), Catcheside (1930) and others is so strong that it can not be lightly set aside.

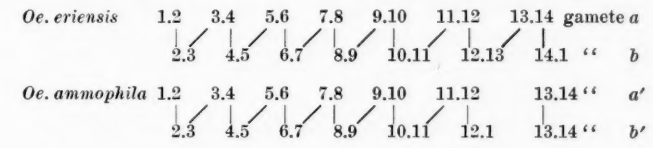
More recently the theory of catenation through translocation or exchange of segments by crossing-over be-

tween non-homologous chromosomes has been receiving increasing attention. There are many things to be said in favor of this view, but it is also not without its difficulties, although as a principle of explanation it appears capable of considerable extension. Blakeslee and Cleland (1930) have recently applied this hypothesis to the ring formation in Oenothera. While I have assumed (Gates, 1928) that linkage has arisen through crossing between species having certain non-homologous chromosomes, they begin with a hybrid between two forms in which certain chromosomes are unlike because an exchange of segments has taken place between two chromosomes in one of the parental forms (in accord with the hypothesis of the origin of the B forms in Datura). This would lead to the formation of a form having a ring of four chromosomes, and so by crosses between this form and others the catenation could be increased until forms would be produced having a ring of 14. The presence of balanced lethals in the chromosomes of the ring is necessary, since the forms must then breed true because the homozygous recombinations will be non-viable.

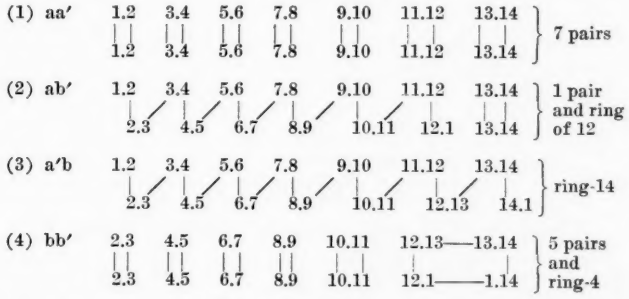
The essential difference between their hypothesis and my former view is that, while we both assume crossing to have occurred, my hypothesis assumed that the spireme fails to segment off the non-homologous (unpaired) chromosomes in a hybrid, while they assume that reversal of ends in a pair of chromosomes has already occurred (before crossing) through segmental interchange between non-homologues. This interchange, for which Darlington also argues on the basis of chiasmas and parasynapsis, would, if it were sufficiently frequent (as in Tradescantia) practically do away with chromosome individuality during the period of meiosis, as already pointed out. But in Oenothera the evidence is all for the fixity of any linkage once it is established, and this being the case segmental exchange between non-homologous chromosomes may be expected to be relatively rare. In

any case, crossing appears necessary for the production of the large number of phenotypically distinct species with different linkages known in Oenothera. In a further paper, Cleland and Blakeslee (1930) make various predictions of the catenation in hybrids between species whose chromosome linkage is already known, and the success of these predictions considerably strengthens this hypothesis.

We may briefly consider certain cases of catenation on the basis of this hypothesis, using a modified notation of Blakeslee and Cleland. Thus we may represent



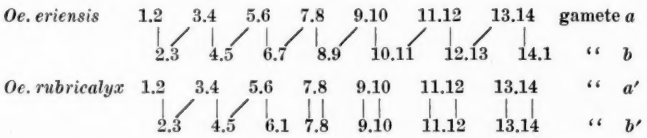
where *eriensis* has a ring of 14, and *ammophila* one pair and a ring of 12. The four possible combinations of complexes would then be as follows:



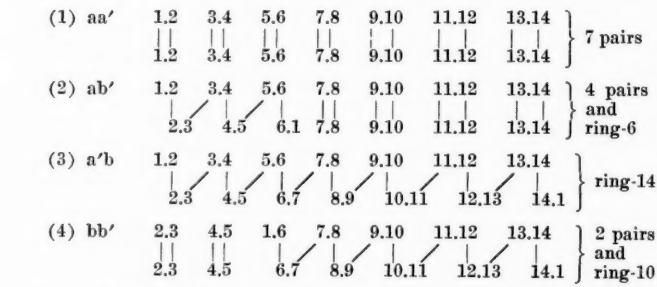
Now *Oe. eriensis* and *ammophila* are closely related. Although their catenation differs, they are so much alike that many botanists would consider *eriensis* a variety or subspecies of *Oe. ammophila,* but their differences remain absolutely sharp in cultures. We must assume that the relatively homozygous recombinations *aa'* and *bb'* are non-viable in the hybrids as they are in the parents, since

these linkages do not occur in the hybrids. This is a reasonable assumption, as the species themselves are both no doubt heterozygous complex-combinations breeding true, as Hoeppener and Renner (1928) have shown to be the case in *Oe. ammophila.* Then there is the further question, why does *Oe. eriensis* × *ammophila* apparently always give one free pair and a ring of 12, like the pollen parent (seven different F_1 plants from three separate crosses were examined, Sheffield, 1929), while the reciprocal (four F_1 plants examined) always has a ring of 14, again like the pollen parent? This must be because in *eriensis* × *ammophila* only the *a* female gametes of *eriensis* function, producing the combination *ab′*, which has a ring of 12 chromosomes. Similarly in the reciprocal only the *a′* megaspore of *ammophila* combines with the *b* pollen grain complex to produce the functional combination with a ring of 14 chromosomes.

In the case of *Oe. eriensis* × *rubricalyx* the result is not so simple, since the F_1 hybrid has a different linkage from either parent. Here we have

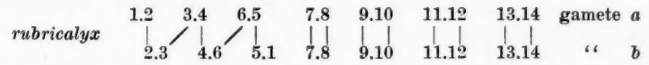


The four possible complex-combinations are as follows:

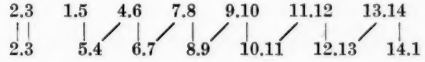


Oe. rubricalyx × *eriensis* produces a crop of seedlings which are non-viable (Gates, 1930). In *eriensis* × *rubri-*

calyx none of the above four types of chromosome catenation appear, but the four F_1 plants examined all showed one pair and a ring of 12. This can only be explained on the basis of the hypothesis by assuming that two of the chromosomes in the ring of six in *rubricalyx* are differently constituted. If we write



in which chromosomes 4.5 and 6.1 are replaced by 4.6 and 5.1, then in a cross with *eriensis* the *bb′* complex-combination would give



showing the desired catenation. In this case it is not unlikely that the *bb′* combination would be viable, since the complexes of which *rubricalyx* is composed are very similar. While there is a degree of artificiality about the method, yet the scheme can be made to "work" by making certain assumptions regarding the constitution and previous translocations of the chromosomes. It is not clear, however, why an *ab′* combination, which on the above constitution of *rubricalyx* would have a ring of 14, should be non-viable. In order for the *ab′* combination to have a ring of 12, a larger amount of rearrangement between chromosome segments would be necessary.

The same situation arises in connection with *Oe. rubricalyx* × *novae-scotiae*. In this case the seed parent has a ring of 6 and the pollen parent a ring of 14, while the F_1 hybrid again shows one pair and a ring of 12.

There is much evidence, both from plant and animal hybrids, to show that the parental chromosomes maintain their morphological individuality in the hybrid soma. The theory of translocation assumes, however, that during meiosis in the hybrid the individuality is more or less broken up. In such genera as Antirrhinum, where interspecific hybrids show Mendelian behavior, no such change is to be expected and no catenation is known. Catenation

will no doubt prove characteristic of certain genera, just as are the various forms of apomictic development.

We may now refer briefly to the cytological implications of the theory of translocations combined with hybridization as the cause of the catenations observed in Oenothera. Darlington has argued with great acuity, from the parasynapsis and chiasma behavior observed especially in various Monocotyledonous families, that the same conceptions must be of universal application, but the facts do not lend themselves easily to this interpretation. As is already well known, there is a heavy weight of evidence for telosynapsis in Oenothera and the evidence seems equally clear in certain other genera, such as Lathyrus. It would be tempting to suppose that Oenothera is parasynaptic, especially as parasynapsis has been described in Godetia (Håkansson, 1925) and Eucharidium (Schwemmle, 1926); but at present the cytological facts will not permit it. If Darlington's conceptions were applicable to Oenothera, then the connections between consecutive chromosomes in the spireme should always be double, but they have invariably been observed to be single. Their smaller size than monocotyledonous chromosomes will not suffice as an explanation of the failure to observe these double connections, for such connections have been seen and figured in the still smaller chromosomes of Pyrus (Darlington and Moffett, 1930).

Some years ago I expressed the view (Gates, 1922, 1924) that telosynapsis was incompatible with crossing-over, such as has been described in certain cases in Oenothera. Since that time, however, evidence has been adduced indicating that, for example, in Lactuca (Gates and Rees, 1921) and Lathyrus (Latter, 1926) a telosynaptic arrangement of chromosome loops could be accompanied by a possible basis for crossing-over, the chromosomes being twisted round each other in the loop stage of the spireme. It follows that telosynaptic pairing does

not exclude the possibility of crossing-over even in Oenothera, but it appears probable that much of such exchange in Oenothera is of whole chromosomes (as originally pointed out in 1908) rather than intra-chromosomal.

Meanwhile the evidence is clear that specific attractions are responsible for the formation of the chromosome rings and chains so characteristic of Oenothera. This evidence has recently been strengthened by the papers of Håkansson (1930*a*) on the catenation in a number of trisomic mutants of *Oe. Lamarckiana* in which the most frequent arrangement is an open chain of 13 and a bivalent. In another paper (1930*b*) he accepts the view that half-mutants such as *rubrinervis* arise through crossing-over between chromosomes widely separated in the ring, but emphasizes the difficulty pointed out by Sheffield (1929) that on this basis many other mutations should arise from *Lamarckiana,* having different linkages from the 4 pairs and ring of 6 found in *rubrinervis.* His *rubrisepala* α, with 5 pairs and a ring of 4, is, however, the only one known.

The term asyndesis has recently been used (Kuhn, 1930) for the omission of chromosome pairing, which is characteristic of the meiosis in haploids. In haploid Oenotheras, Davis and Kulkarni (1930) and Emerson (1929) have pointed out that the spireme stages up to diakinesis are the same as in the diploid, *i.e.,* single threads in both. Weier (1930) has recently drawn a contrast between the conditions of the meiotic prophase in *Oe. Hookeri* with 7 chromosome pairs and *Oe. Lamarckiana* with a ring of 12. He finds parasynaptic pairing of threads in *Hookeri,* but not in *Lamarckiana* except as regards the one pair. He thus agrees that parasynapsis does not occur in *Lamarckiana* as regards the 12 chromosomes in the ring, but he calls the condition asynapsis. There appears to be no advantage in thus altering the antithesis between parasynapsis and teleosynapsis which cytologists have universally recognized for many years.

As regards Weier's observations themselves, the fixation of his material is far from satisfactory, as his own statements show. He repeatedly writes of and figures a "central coagulum" in the nucleus, which other critical investigators of the cytology of Oenothera have not found in their preparations. He believes that he finds in *Hookeri* a zygotene stage of pairing leptonema threads such as is not found in *Lamarckiana,* but his evidence on this point leaves much to be desired. Later, in the pachynema stage, he finds in *Hookeri* seven loops, but makes the old mistake of interpreting the two approximated arms of each loop as due to a split in the thread itself, separating the leptotene halves. This stage in which seven loops are described corresponds roughly with the brochonema stage in Lathyrus (Latter, 1926) although the arrangement of the loops is by no means so clear in Oenothera. Finally, in the second contraction stage, Weier recognizes "seven bivalent arms" projecting from the central coagulum, which he interprets as due to the "shortening and thickening of the seven bivalent threads," although the obvious interpretation appears to be that each of the "seven bivalent arms" is a pair of chromosomes end-to-end.

According to this account, *Lamarckiana* differs in having no zygonema stage except for the one pair of chromosomes. But most remarkably, in the pachynema stage he claims to find 13 arms (not loops) projecting from the "central coagulum," one of them being double and representing the free pair of chromosomes. Equally remarkable is the statement that these 13 free arms later join up by their projecting ends to form a continuous pachynema, before undergoing the second contraction and segmenting into chromosomes as described by previous investigators. In short, this paper supplies what are believed to be the theoretical cytological requirements of the moment, but there is very little evidence to substantiate his conclusions where they run contrary to previous results. We

agree with the author that in such Oenotheras as *Lamarckiana,* which forms rings of chromosomes, the homologues, if such can be said to exist, fail to mate. This view, however, is not new, but has been generally held for many years.

The genus Oenothera has served as the basis for many new conceptions in the history of genetics, and its prolificacy in that regard is probably not yet at an end. Such differences of interpretation as have existed have led always to new knowledge, and there is no doubt that the same will be true in the present instance. There is, however, a danger in making cytological observations too subservient to genetical theory, and it is for this reason that I refrain from accepting, without adequate cytological evidence, interpretations which, if true, would form an easy road through a considerable jungle of fact.

Summary

The term mutation should be used in a generic sense to include inherited changes of any kind in the germplasm. Various types of mutations, such as trisomics, polyploids, translocations and gene mutations, can then be classified.

The remarkable constancy in the structural arrangement and spatial relationships of the elements making up the germinal material under normal conditions is emphasized.

The term *catenation* is proposed for the linkage of chromosomes in a ring or chain, now known to occur in a number of plants.

The causes of catenation, especially in Oenothera, are discussed, and the theory of hybridization combined with some translocations between non-homologous chromosomes is supported.

The present cytological evidence for parasynapsis in Oenothera is not regarded as satisfactory, although if parasynapsis occurred it would afford a basis for much at least of the chromosome ring formation in Oenothera.

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OENOTHERA LAMARCKIANA MUT. *ACUTIFOLIA*, A NEW MUTANT TYPE PRODUCED BY A GENE OUTSIDE THE FIRST LINKAGE GROUP

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IN the forty-odd years in which *Oenothera Lamarckiana* has held the attention of students of heredity it has demonstrated itself to be very singular in its behavior. Although mutations have appeared in comparatively large numbers, the vast majority of these have been "anomozygous" mutations (caused by specific irregularities in chromosome distribution) and "crossover" mutations (Shull, 1921). Mutations of these types recur year after year in experimental cultures of *Oe. Lamarckiana*. On the other hand, demonstrated gene mutations have arisen with extreme rarity during the extensive study of this species. Of these demonstrated gene mutations, only four have been shown to be inherited in typical Mendelian manner; that is, giving a 3:1 ratio by the selfing of a heterozygote and a 1:1 ratio by the backcrossing of the heterozygote to the recessive type. The other mutations have given peculiar ratios because of their linkage with the balanced lethals found in linkage-group I.

Of the four gene mutations referred to above which gave typical monohybrid ratios, one, the short-styled factor of *brevistylis* (b_r), was found by deVries in wild stock and has not appeared *de novo* in pedigreed cultures. The three remaining gene mutations behaving in typical Mendelian manner arose in the pedigreed stock of *Oe. Lamarckiana* of Dr. George H. Shull, of Princeton University. These are: Old-gold flower color of mut. *vetaurea* (v); double flowers of mut. *supplena* (s_p), and mut. *bullata* (b_u), the latter modifying the entire aspect of the plant but most strikingly characterized by intense crinkling of the leaves (Shull, 1925, 1926, 1928*a*).

This paper reports the occurrence of a new gene mutation in pedigreed *Oe. Lamarckiana* and gives data to show that it is the fifth gene mutation to give typical monohybrid ratios on self-fertilization of the heterozygotes. As a name descriptive of the most obvious manifestation of the new gene, *Oe. Lamarckiana* mut. *acutifolia* (a_f), is proposed.

DESCRIPTION OF *Oe. Lamarckiana* mut. *acutifolia*, mut. nov.

The most outstanding and consistent diagnostic character of mut. *acutifolia* is the pointed apex of the rosette leaf. Usually, at the very tip of the blade the edges are perfectly straight, coming to a fine point; in some cases the distal edges of the rosette leaf actually present a concave, recurved aspect (Fig. 1). By way of contrast the

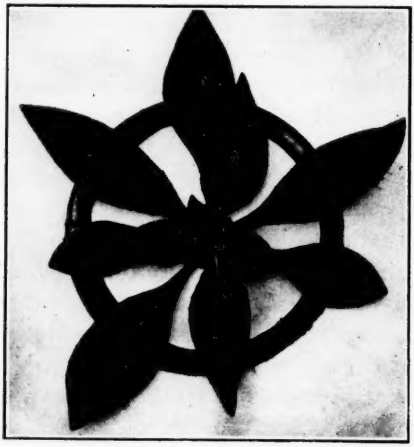

FIG. 1. A nine weeks old rosette of *Oenothera* mut. *acutifolia*.

typical *Oe. Lamarckiana* leaf curves convexly throughout its entire length, and acutely pointed tips such as are present in the new form are rare (Fig. 2).

FIG. 2. A nine weeks old rosette of *Oenothera Lamarckiana*.

Another feature of the rosette leaf of *acutifolia* is its reduction in width. It is quite consistently narrower than the typical *Lamarckiana* leaf. The actual length remains the same, although relatively, because of the decrease in width, the mutant rosette leaf seems longer and more graceful.

The crinkling which appears prominently even in very young *Lamarckiana* rosettes is almost completely lacking in *acutifolia* rosettes of the same age. At a later stage, as the crinkling increases in *Lamarckiana* rosettes, the rosettes of the mutant develop a conservative amount of crinkling, although rarely equaling that manifest in rosettes of the former.

Oe. acutifolia is a weaker plant and of slower growth than *Oe. Lamarckiana*. The mutant plant, whether it be rosette or mature, is quite consistently the smaller and less vigorous. As the plants in the field approach maturity those of *acutifolia* are uniformly later by weeks in blooming. Quite an interesting feature is that the mutant plants are extremely tardy in sending up central

vertical stalks. The flowering for some time takes place exclusively on semi-procumbent side branches. Many *acutifolia* plants never develop a vertical stalk.

No apparent differences in the color of the foliage and the texture are to be found between *Oe.* mut. *acutifolia* and its parent species. Also the dark red spots which are so characteristic of the rosette leaf of *Oe. Lamarckiana* occur with similar frequency and degree on the rosette leaf of the mutant *acutifolia.* This new form closely parallels its parent species in floral characters and breeding phenomena. The morphological features of the flowers, the amount and fertility of the pollen, the seed output and the percentage of germination are the same in both forms.

ORIGIN OF mut. *acutifolia*

In the summer of 1928 there was placed in my hands a family (S274) of Evening Primroses, the members of which were phenotypically *Oe. Lamarckiana.* This family was a unit in a line that had been maintained for twenty-three years by Dr. Shull by appropriate crosses so that the parents of each family in the line were in no case related more closely than second cousins.

At the time, some irradiation work was being conducted in this family of *Lamarckiana,* and five plants were chosen to provide control progenies. These five plants were self-fertilized and their five progenies grown the following spring (1929). As the rosettes developed full expression of characters it was found that the progenies from three of the five plants were segregating in a 3:1 ratio for the wholly new type of rosette that forms the subject-matter of this report. The other two plants threw typical *Lamarckiana* offspring. The details are shown in Table I.

Three segregating plants out of the five tested suggested strongly that the family of *Oe. Lamarckiana* turned over to me in 1928 consisted of homozygous and

TABLE I

SELFINGS IN *Oe. Lamarckiana* FAMILY S274, THREE PLANTS OF WHICH THREW PROGENIES WHICH SEGREGATED FOR THE NEW MUTANT, *Oe. acutifolia*

PEDIGREE NUMBERS		Population	Non-*acutifolia*	*Acutifolia*
Plant	Family			
S274(27)	2823	89	89	0
S274(29)	2830	67	67	0
	Total.....	156	156	0
S274(20)	288	80	59	21
S274(26)	2815	66	52	14
S274(33)	2837	64	54	10
	Total.....	210	165	45
	Theoretical 3:1		157.5	52.5

heterozygous individuals, indicating that the *acutifolia* gene probably arose by a mutation which occurred in 1927, the heterozygous plant thus produced having been mated with a normal *Lamarckiana* in maintaining the cross-bred line. That this mutation did not exist in a latent condition in the several families of the cross-bred line has been shown by a series of crosses made during the summer of 1929 by Dr. Shull. Plants from five of the eight families constituting his cross-bred *Oe. Lamarckiana* strain were pollinated by mut. *acutifolia.* In each case, the F_1 progeny grown in 1930 has consisted of *Lamarckiana* and its ordinary known mutants not including mut. *acutifolia.*

BREEDING TESTS WITH *Oe.* mut. *acutifolia*

As shown by Table I, family 288 segregated for *acutifolia* in a fair approximation to the theoretical 3:1 ratio. This group of plants was chosen as material with which to investigate more fully the breeding behavior of *acutifolia.*

Three plants of mut. *acutifolia* were selfed to determine the pure-breeding habit of the new form. Table

II gives the results of these breedings. The three progenies totaled 71 plants; 66 were unmistakably *acutifolia,* 2 were unidentified variants such as commonly occur in *Lamarckiana* and its derivatives, and 3 were doubtful plants because of aphid attack. It may safely be said, therefore, that *acutifolia* is a pure-breeding type.

TABLE II

SELFED *Oe.* mut. *acutifolia,* SHOWING THE PURE-BREEDING CHARACTER OF THIS NEW MUTATION

PEDIGREE NUMBERS		Population	*Lamarckiana*	*Acutifolia*	Other variants	Unclassified (diseased)
Parent	Family					
288(17)	29181	21	0	20	0	1
288(28)	29182	12	0	11	0	1
288(33)	29183	38	0	35	2	1
	Total.....	71	0	66	2	3

Further investigation with family 288 consisted of breeding from the phenotypic *Lamarckiana* plants, numbering 59, to ascertain if their genetic constitutions were of two kinds, namely, A_fA_f and A_fa_f. To do this, 31 typical plants were selected at random and either self-fertilized or crossed with *acutifolia.* With two plants, 288(35) and 288(36), both self-fertilizations and backcrosses were made, so that as a total 23 selfings and 10 backcrosses were accomplished; in the backcrosses, *Lamarckiana* was used as female parent in three crosses, as male parent in seven crosses. No differences appeared in families from reciprocal crosses.

In a segregating family such as 288, the theoretical genotypic ratio for the *Lamarckiana* plants is 1:2. Accordingly, one third, or 10.33, of the 31 plants should be homozygous (A_fA_f) and two thirds, or 20.67, should be heterozygous (A_fa_f). Actually, eight plants of the 31 bred true for *Lamarckiana,* while 23 threw progenies which segregated for *Lamarckiana* and *acutifolia* either in the 3:1 ratio or the 1:1 ratio, depending on the method

of breeding. There is a departure of 2.33 plants from the theoretical, as close a fit as the small number of plants tested could be expected to give.

The data from these selfings and backcrosses are contained in Tables III, IV and V.

Table III is a tabulation of the crosses between *acutifolia* and A_1a_1 *Lamarckiana* of family 288. The number of rosettes grown was 235, of which 17 were so badly attacked by aphids as to render classification insecure. Of the 218 that could be classified 112 were non-*acutifolia* and 106 *acutifolia*. When the theoretical ratio of 109 : 109 is considered, the actual ratio is a remarkably close fit. On a unity basis, with the theoretical 1 : 1, the actual ratio is 1.05 : 1.

TABLE III

CROSSES OF *acutifolia* AND A_1a_1 *Lamarckiana* PLANTS IN SEGREGATING FAMILY 288

PARENTS		Family	Population	Non-*Acutifolia*	*Acutifolia*	Unclassified (diseased)
Female *Acutifolia*	Male *Lamarckiana*					
288(15)	288(12)	291	13	4	9	0
288(15)	288(13)	292	2	1	1	0
288(15)	288(60)	293	9	3	6	0
288(15)	288(60)	29186	19	8	10	1
288(26)	288(75)	295	3	2	1	0
288(28)	288(36)	296	5	2	3	0
288(28)	288(36)	29188	5	3	2	0
288(28)	288(37)	297	8	2	6	0
288(28)	288(37)	29189	29	14	12	3
Lamarckiana	*Acutifolia*					
288(43)	288(22)	298	13	6	7	0
288(43)	288(22)	29190	35	16	12	7
288(47)	288(15)	299	6	1	5	0
288(47)	288(15)	29191	5	4	1	0
288(48)	288(22)	2910	16	12	4	0
288(48)	288(33)	2911	11	6	5	0
288(48)	288(33)	29192	56	28	22	6
Total.....	235			112	106	17
Expected 1 : 1				109	109	

The progenies from sixteen self-fertilized heterozygotes are recorded in Table IV. Of 887 rosettes comprising the total population, many plants suffered from the attacks of aphids and 37 had to remain unclassified. The remaining 850 plants split into 668 non-*acutifolia* : 182 *acutifolia*. On a 3 : 1 basis, the numbers should have been 637.5 and 212.5, respectively. The actual ratio reduces to 3.67 : 1.

TABLE IV

SELFED $A_f a_f$ *Lamarckiana* IN SEGREGATING FAMILY 288

PEDIGREE NUMBERS		Population	Non-*acutifolia*	*Acutifolia*	Unclassified (diseased)
Parent	Family				
288(36)	29194	45	37	8	0
288(37)	29195	39	31	6	2
288(39)	29196	50	32	8	10
288(40)	29197	62	45	14	3
288(41)	29198	17	12	5	0
288(46)	29199	56	47	9	0
288(51)	29200	33	27	6	0
288(52)	29201	79	56	20	3
288(56)	29203	116	93	21	2
288(58)	29205	69	54	14	1
288(61)	29207	70	54	16	0
288(63)	29209	43	33	10	0
288(69)	29213	64	42	10	12
288(73)	29214	24	17	7	0
288(74)	29215	59	41	18	0
288(77)	29216	61	47	10	4
Total.....		887	668	182	37
Expected 3:1			637.5	212.5	

Table V contains the individuals in the segregating family 288, which by breeding results were proved to have the genetic constitution $A_f A_f$. The progenies of nine plants are represented in the table. The total population is 530 plants, 21 of which are mutants and variations common to *Lamarckiana* pedigrees. The remainder (509 plants) consist of 507 rosettes of *Oe. Lamarckiana* and two rosettes of *acutifolia*. These last-

named undoubtedly represent contamination at some stage in the cultural operations attending the growing of the plants. They need not affect the validity of the families in which they appear.

TABLE V

A_fA_f *Lamarckiana* PLANTS IN SEGREGATING FAMILY 288. (ALL FAMILIES EXCEPT 294 AND 29187 ARE DERIVED FROM SELFINGS.)

PARENTS		Family	Population	*Lamarckiana*	*Acutifolia*	Other variants
Female	Male					
288(17)	288(35)	294	23	22	0	1
288(17)	288(35)	29187	40	39	0	1
288(35)	self	29193	32	31	0	1
288(53)	"	29202	64	63	0	1
288(57)	"	29204	104	100	0	4
288(59)	"	2930	12	8	0	4
288(59)	"	29206	9	8	0	1
288(62)	"	29208	80	78	1*	1
288(65)	"	2935	8	7	0	1
288(65)	"	29210	32	31	0	1
288(66)	"	29211	47	44	0	3
288(68)	"	29212	79	76	2*	2
		Total.....	530	507	2*	21

* Undoubtedly contamination.

All families in Table V except 294 and 29187 were derived from self-fertilizations. These particular families, 294 and 29187, may be cited as showing the complete recessiveness of mut. *acutifolia,* as the parents of each were *Lamarckiana* (proved to be (A_fA_f) and *acutifolia* (a_fa_f). The F_1's (A_fa_f) are as follows: Family 294 consists of 23 plants, all *Lamarckiana* as the variant listed in the table is merely a normal *Lamarckiana* rosette with two growing points; family 29187 consists of 40 plants, 39 of which are *Lamarckiana,* the variant listed being *Oe.* mut. *pulla* (one of the recurring trisomics).

DISCUSSION

The breeding results presented in the foregoing tables establish the following points concerning the heredity of

Oe. mut. *acutifolia*: (1) that the characters of this new form are the expression of a single gene, a_f, which arose by a mutation presumably in 1927; (2) that *acutifolia* is a pure-breeding type; (3) that it is completely recessive when mated with its parent species, *Oe. Lamarckiana;* (4) that a typical 3:1 ratio is obtained in the progeny of a selfed heterozygote (A_fa_f) of *Lamarckiana* phenotype, and (5) that the dominant type in a segregating family can be identified as homozygotes and heterozygotes in about the typical 1:2 ratio.

These facts show that mut. *acutifolia* is included among the small group of mutations of *Oe. Lamarckiana* which segregate in typical monohybrid ratios. This feature serves definitely to place gene a_f outside of the extensive linkage-group I, since genes in this group are prevented from appearing with the typical Mendelian frequency because of their association with the balanced lethals, l_1 and l_2, characteristic of *Lamarckiana* (Shull, 1923). There are three possibilities relative to the linkage relationship of mut. *acutifolia* with other known genes: (1) either the gene for *acutifolia* is linked with *brevistylis* (b_r) in linkage-group II; (2) it is linked with *vetaurea* (v), *supplena* (s_p), and *bullata* (b_u) in linkage group III, or (3) it segregates independently from the above four genes and is thus the first recognized gene of a new linkage-group IV. The determination of the linkage relations of mut. *acutifolia* is under investigation by Dr. Shull.

The importance of finding additional genes in *Oe. Lamarckiana* can not be too strongly emphasized, for it is through these that the solution of the cytological-genetical puzzle in this species will come. As is well known from the brilliant work of the Oenothera cytologists, telosynapsis is quite commonly present in Oenothera species, and in *Oe. Lamarckiana* twelve of the chromosomes at diakinesis remain attached together and only one free pair is present. Two conflicting lines of thought have arisen in the attempt to interpret the relationship be-

tween this extensive linkage and the factor linkage as found in *Lamarckiana.*

Cleland (1929), and the majority of *Oenothera* cytologists and geneticists associate the extensive linkage-group I with the circle or chain of twelve chromosomes and postulate a theory which forms a basis for only two linkage-groups in a form with a circle of twelve chromosomes and a single pair.

Shull (1928*b*), on the other hand, believes that the chromosome circle in *Lamarckiana* need not be the basis for only one linkage-group. He does not fall into line with the essential part of the theory of Cleland and others who argue that the chromosomes are arranged in a definite order in the chain, that is, alternately maternal and paternal. Shull believes that the orientation of the chromosomes within a single homologous pair is entirely a matter of chance, but that the pairs maintain their identity in such manner that material and paternal members of the same pair are normally separated at meiosis. According to the latter view, it is quite possible to have as many linkage-groups as there are haploid chromosomes. That is, *Oe. Lamarckiana,* with its circle of twelve and one pair, could segregate for seven linkage-groups, instead of only two.

The determination of these linkage-groups will determine the answer to this cytological-genetical problem, hence the great importance to be attached to new genes which are free from the first linkage-group.

The cytology of *Oe.* mut. *acutifolia* has been investigated through a study of the stages of meiosis in the pollen mother cells. The 2n number has been found to be fourteen, as in the parent species, in accord with the genetical indications that mut. *acutifolia* represents a gene mutation. In addition, there are indications that the chromosomes of *acutifolia* in diakinesis arrange themselves in a circle or chain of twelve with one free pair. Work is now in progress to confirm this preliminary statement. With a circle of twelve and one free

pair mut. *acutifolia* will thus have the same chromosome configuration as its parent species, *Oe. Lamarckiana.*

SUMMARY

1. *Oenothera* mut. *acutifolia* appeared in the spring of 1929 in a cross-bred line of *Oe. Lamarckiana* which had been maintained for twenty-three years.

2. This new gene mutation is characterized by a narrower, more sharply pointed rosette leaf and a reduction in the amount of crinkling. Plants of *acutifolia* grow much more slowly than typical plants of *Lamarckiana* and are several weeks later, on the average, in coming to bloom.

3. When *acutifolia* first appeared it made up 21.4 per cent. of the population of three segregating families.

4. Self-fertilized *acutifolia* has bred true.

5. In a segregating family, 31 phenotypic *Lamarckiana* plants were tested and shown to be of two genetic constitutions: Eight plants (A_fA_f) gave typical *Lamarckiana* progenies; twenty-three plants (A_fa_f) threw segregating families.

6. *Acutifolia* is completely recessive. When it was crossed with A_fA_f *Lamarckiana,* no *acutifolia* appeared in the F_1; when it was crossed with *Lamarckiana* heterozygous for *acutifolia* a 1:1 segregation resulted.

7. The occurrence of typical monohybrid ratios in self-fertilized *Lamarckiana* indicates that the gene for *acutifolia* (a_f) is not in linkage-group I with the balanced zygote lethals. Either it is in group II with b_r; or in group III with v, s_p and b_u; or in group IV for which it would be the first known gene.

8. Mut. *acutifolia* has fourteen chromosomes (2n), which at diakinesis appear to be arranged in a circle or chain of twelve and a single pair. It seems, therefore, that the configuration in *acutifolia* is identical with that found in its parent species, *Oe. Lamarckiana.* The cytological work with this form is being continued.

9. The importance of new genes in *Lamarckiana* which give typical Mendelian ratios is stressed as providing the means towards solving the problem of the true significance of chromosome cohesion in Oenothera species.

It is a pleasure to acknowledge my indebtedness to Dr. George H. Shull, under whose guidance my work with Oenothera is being done, for facilities for the execution of this work, for the original pedigreed material and for his interest and helpful suggestions and criticisms which have been invaluable.

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THE EFFECTS OF INCREASING X-RAY VOLTAGES ON THE PRODUCTION OF LETHAL MUTATIONS IN DROSOPHILA MELANOGASTER[1]

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THAT X-rays and radium induce mutations in relatively large numbers is now well established. New discoveries give rise to new problems, and the question now has become: What is the relation between the physical agent, whether X-rays or radium, and the resulting biological phenomena? More specifically, the problem concerns the effect of increased dosage on the frequency of mutation.

In work with Drosophila it has become apparent that the number of visible mutations occurring can be used only as a rough indication of the gene-mutation rate, as these are relatively infrequent and difficult to detect. For true analysis the more objective lethal-mutation methods, especially the *C1B* technique of Muller, have proved much more satisfactory.

Early in his studies of mutations in irradiated Drosophila, Muller (1928) used two dosages in which the only variable was duration of exposure. These dosages are designated in Muller's laboratory as T_2 and T_4, the latter being twice the strength of the former. Results based on the percentages of lethals showed that "an increase in X-ray dosage causes an increased production of mutations," though Muller did not consider the numbers of these early experiments sufficiently large for an exact ratio.

Weinstein (1928) secured from the use of Muller's two dosages results substantiating Muller's own. Wein-

[1] The expenses of this investigation were supported in part by the Committee on the Effects of Radiation upon Living Organisms of the National Research Council.

stein's figures include a number of lethals and visibles which again suggest that a greater effect is obtained by means of a stronger dosage.

Hanson, also in 1928, interested particularly in the effects of the T_2 and T_4 treatments on productivity and the sex ratio in Drosophila, incidentally made counts of visible mutations which indicate a definite relation between their number and the dosage. His data (1928b) on the number of return mutations from bar to round indicate a similar relation.

Oliver (1930) has made the relation of the mutation rate to dosage the primary purpose of a series of experiments. From the use of five X-ray dosages he concluded that "the total number of lethals is directly proportional to the dosage used when the only factor varied is the duration of the treatment."

In all the foregoing experiments dosages were used which differed only as to the time factor. A new approach to the general problem was made by Hanson and Heys (1928, 1929), who varied the intensity of radium rays by means of a series of filters. Their results show that the percentage of lethal mutations in Drosophila varies directly with the thickness of the filter, and is proportional to the amount of ionization produced in air.

It is of interest to note that evidence of a relation between dosage and mutation frequency has come also from experiments with plants. Stadler (1930) used visible seedling mutations in barley as an index of the mutation rate, and found, when he varied the time of exposure of treated seeds, that the "mutation frequency increased approximately in direct proportion to dosage." He studied as well the effect of voltages within the limits 40 to 116 kilovolts. The X-rays of different wave-lengths thus produced appeared "to be about equal in power to cause mutation when applied in intensities equal in power to ionize air."

Thus a number of different factors have been investigated. On the one hand it seems reasonable to conclude

that wave-length or *quality* of X-rays has no effect on the mutation process; while other experiments give definite evidence of a relation between mutation and the dosage or *quantity* of radiation. Since dosage is the product of two factors, time and intensity, it must be considered from two angles. In the case of radium treatment, the number of mutations appears directly proportional to the intensity, while two different experiments with X-rays indicate that the number of mutations varies directly with the duration of exposure.

The present investigation was concerned with still another factor, X-ray intensity. The question in mind was this: When X-ray intensity is varied by using treatments which differ only as to the voltage across the X-ray tube, how is the mutation process affected?

EXPERIMENTAL PROCEDURE

The so-called *C1B* stock was used in these experiments. In this strain two groups of mutant, sex-linked genes are present. One X-chromosome contains the series of recessive characters, scute (sc), vermillion (v), forked (f) and bobbed (bb). The other contains the dominant *C* factor which prevents crossing-over, a recessive lethal (1), and the dominant gene for bar eye. One type of male and two types of females are viable. All the males have the *scvfbb* chromosome. Half the females are homozygous for these characters. The others have one *scvfbb* chromosome and one *C1B* chromosome. The first group of females shows round eyes, while the second has the characteristic heterozygous, kidney-shaped bar eye.

Heterozygous bar-eyed females were mated in pairs to wild X-rayed males. The F_1 females are bar and non-bar. The bar-eyed females were mated to their brothers, one pair in a tube, to produce the F_2 generation. The F_1 *C1B* female receives a *C1B* chromosome from her mother and a treated X-chromosome from her father. She gives to one half her sons the lethal *C1B* chromo-

some, and to the other half the treated chromosome. Due to the presence of the lethal half the sons fail to hatch. If a new lethal mutation were induced in the treated chromosome, the other half of the sons would inherit it and also fail to appear. Therefore, by examination of the F_2 cultures, it was possible to detect a lethal mutation in the X-chromosome which occurred in the male treated two generations before. To determine the number of lethal mutations it was necessary only to record the number of cultures which hatched 100 per cent. female flies.

In eleven different X-ray treatments the voltage was varied from 40 to 99 kilovolts. All other factors remained constant: tube current 5 M.A.; target distance 20 centimeters; filter, one millimeter of aluminium; and time, 28 minutes. Before and after each treatment ionization values were read from a Victoreen dosimeter. For convenience, the ionization is recorded as the reciprocal of the time in seconds which it takes an indicator to move across 1,000 divisions of the dosimeter scale. To obtain intensity values in r units per unit minute, it is necessary only to multiply by a constant k the value of which is 1,000.

Experimental Results

The results given in Table I represent a total of twelve experiments in which eleven different dosages were used. The 95-kilovolt experiment was repeated, since the results obtained seemed inconsistent with those of the other experiments. A second trial gave data consistent with the rest.

It is seen readily from the table that both the amount of ionization and the percentage of lethal mutations increase as higher voltages are applied to the tube. Since voltage as such was not measured, the relation between voltage and mutation is of no consequence here. The essential fact for the problem in question is the correlation between mutation and ionization, which is the measure of intensity. In Fig. 1 this relation is shown

TABLE I

SHOWING THE RESULTS OF THE ELEVEN DIFFERENT DOSAGES: VOLTAGE VARIED AS INDICATED; 5 M.A.; 20 CENTIMETERS DISTANCE; 1 MILLIMETER ALUMINIUM SCREEN; TIME, 28 MINUTES. 40 KV DOSAGE APPROXIMATELY EQUALS 445 *r* UNITS; 48 KV – 675 *r* UNITS, ETC.

Dosage X-ray voltage	Ionization proportional to	No. fertile F_2 cultures	No. lethal mutations	Percentage lethal mutations
40 KV	0.0159	799	12	1.502 ± 0.2902
48 KV	0.0241	746	14	1.876 ± 0.3341
52 KV	0.0285	710	13	1.831 ± 0.3394
60 KV	0.0455	607	17	2.801 ± 0.4516
67 KV	0.0588	1097	60	5.469 ± 0.4630
70 KV	0.0555	804	27	3.358 ± 0.4285
76 KV	0.0555	591	32	5.414 ± 0.6278
80 KV	0.0714	636	44	6.918 ± 0.6818
88 KV	0.0689	524	41	7.824 ± 0.7962
a) 95 KV	0.0925	770	48	6.234 ± 0.5877
b) 95 KV	0.1111	747	68	9.103 ± 0.7107
99 KV	0.1111	546	52	9.524 ± 0.8473
Control[2]		1308	1	0.076 ± 0.0515

graphically. The points are plotted as lines, their limits enclosing the ranges of the probable errors. The actual points are cross-bars in the center of each line. The control percentage of mutation, 0.0764 ± 0.0515, is taken from an experiment recently performed in this laboratory for which the same *C1B* method was employed.[3]

It will be noticed that one of the 95-kilovolt experiments and the 99-kilovolt experiment both gave an ionization value of 0.1111. They gave also percentages of mutation differing only by forty-two one-hundredths of one per cent., which may be considered a very small amount in such experiments as these. This agreement seems to the authors to establish rather firmly the upper limits of the curve. Now if a straight line which has its origin

[2] Frank Blair Hanson and Florence Heys, "A Possible Relation between Natural (Earth) Radiation and Gene Mutations," *Science*, 71: p. 44, 1930.

[3] Frank Blair Hanson and Florence Heys, "A Possible Relation between Natural (Earth) Radiation and Gene Mutations," *Science*, 71: p. 44, 1930.

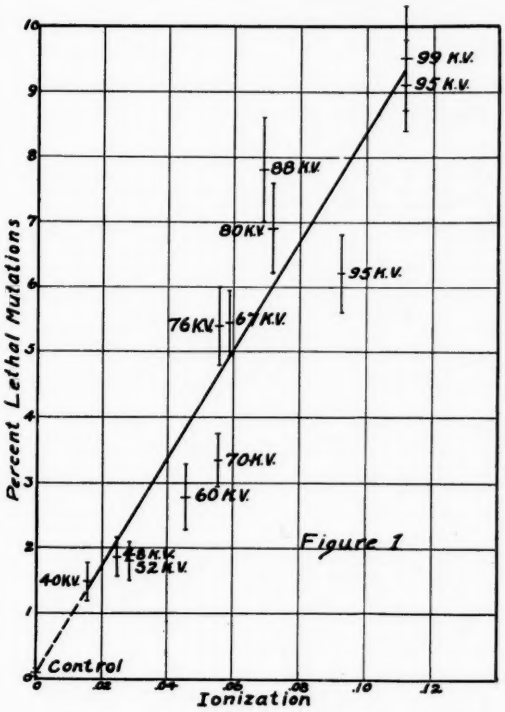

FIG. 1

between these two points is drawn so as to pass through the control point, it is apparent immediately that one half of the points fall on either side. The individual points vary rather widely from the line, but the amount of deviation in one direction is approximately balanced by that in the opposite direction. For a number of reasons this deviation is to be expected. First, the probable errors are for the most part unavoidably large. Numbers for each individual experiment can not be increased greatly beyond the point they have already reached because of the attendant difficulty in handling the flies. Second, the X-ray machine can not be considered a source of constant radiation. There may be fluctua-

tions from time to time either in current or in voltage. Over a twenty-eight minute period of treatment, these changes might be considerable.

The fact that the number of mutations occurring represents a linear function of ionization may be shown by gathering the points into groups arbitrarily, each group representing approximately 10 KV. When these results are averaged the following figures are obtained.

TABLE II

Group	Voltage in KV.	Ionization proportional to	Percentage of mutations	Point
1	40, 48, 52	0.0228	1.753	1
2	60, 67	0.0522	4.135	2
3	70, 76, 80	0.0608	5.230	3
4	88, 95a, 95b, 99	0.0959	8.171	4

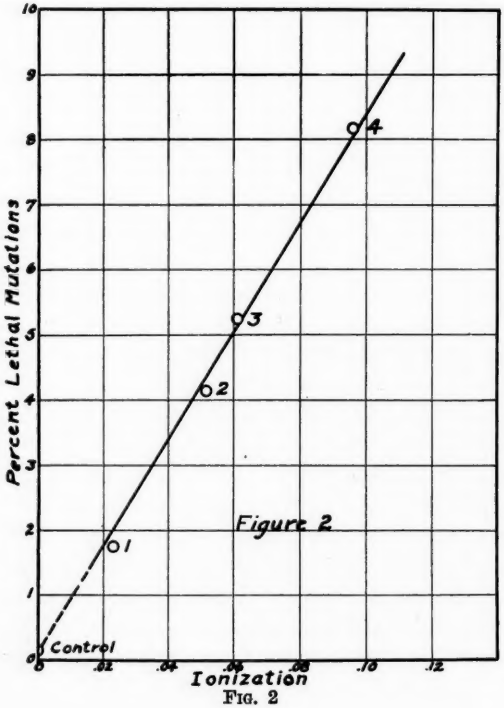

FIG. 2

Fig. 2 shows this relationship graphically. Here the up-and-down deviations of the individual points have been smoothed out by taking the average of arbitrary groups of points which cover a short range of voltage.

Discussion

From the standpoint of physics an increase in X-ray voltage is known to have two effects. First, it increases the proportion of short wave-length or *hard* rays, thereby indirectly increasing the penetrating power of the X-ray beam. Second, a rise in X-ray voltage causes a speeding up of the electrons which pass from the cathode and strike against the target of the X-ray tube. Thus the intensity of the X-ray beam is increased as higher voltages are applied to the tube.

Work by Wood (1924, 1925) on the death-rate of tumor cells, by Packard (1927, 1929) on the death-rate of Drosophila eggs, and by Stadler (1930) on barley mutations gives evidence that wave-length within the limits ordinarily used has no effect. Accordingly, wave-length was not taken into account in this investigation. From the results this seems justifiable, for here again, as in other experiments, no effect of wave-length is indicated.

The relative intensity was determined by methods which utilize a secondary property of X-rays, the ionization of gases. When X-rays strike matter, beta particles or electrons are knocked out from the molecules. The beta particles are moving so rapidly that they *also* may break down other molecules into pairs of ions. Most of the ionization is said to be due to this secondary beta radiation.

Two properties of X-rays have been studied in these experiments. It is known that X-rays produce ionization by means of beta particles. Since the amount of ionization is approximately proportional to the number of mutations, it seems reasonable to suppose that the beta rays which produce ionization are likewise responsible

for mutations. This theory was advanced first by Hanson and Heys (1928, 1929) whose results from experiments with radium radiation showed an almost perfect correlation between ionization and mutation. The results with X-radiation are similar though not quite so definite. The difference in the results of the X-ray and radium experiments, while not a fundamental one, is readily understood when the differences between the two sources of radiation are considered.

SUMMARY

(1) Sex-linked lethal mutations furnish good material for a quantitative analysis of the mutation process.

(2) When the intensity of the X-ray beam is increased by raising the voltage over a range of 40 to 99 kilovolts, the number of mutations is increased accordingly. Taking the amount of ionization in air as a measure, the mutation rate seems to vary approximately in direct proportion to the intensity.

(3) There is some evidence that gene mutations are due not to a number of causes, but to one, the beta particle, and that it is because X-rays and radium both produce beta radiation that they are successful agents in inducing gene mutations in the laboratory. Similarly, it is suggested that some kind of earth radiation producing beta particles may be responsible for mutant types in nature. Recent measurements by Muller and Mott-Smith (1930), however, seem to indicate that natural radioactivity is far "inadequate to explain the frequency of 'natural' mutations."

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INTERNAL FACTORS AFFECTING DISCONTINUITY BETWEEN SPECIES[1]

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MAY I preface my remarks by repeating my conviction that the species problem *is* a problem and that as one of the fundamental problems of biology it is worthy of study as an end in itself and not as a mere corollary to work in ecology, systematics or genetics? Not until a fairly large number of species have been carefully and methodically studied over their entire range shall we be able to discuss the problem intelligently.

Aside from the work on cultivated plants by Vavilov and his associates, there are not even complete morphological surveys of any of the higher plants. For what species can the following simple questions be answered: What is its actual distribution? Does it grow in different situations in different parts of its range? What, for some easily measured character such as height or flower size, are the largest and smallest values for the species, what the average for the species as a whole? Do the values for different localities depart significantly from the mean of the species? Over what part of its range is it the most variable, over what part the least? Are there distinctive color forms and if so what is their comparative frequency in different localities? Are there any geographical regularities in the distribution of such conspicuous characters as hairiness, prostrate habit, etc., and if so, are they paralleled in other species having approximately the same distribution?

For the past six years I have been attempting to gather data which would answer just such questions. I have been studying a few species of Iris, the genus Aquilegia and several species of Aster. While the work is far

[1] Read at the joint discussion on "The Species Concept" at the Fifth International Botanical Congress, Cambridge, August, 1930.

from complete it has progressed far enough to convince me that species (though they are fundamental biological units) are of quite a different nature in these three genera. By this I mean that the morphological resemblances and genetic connection between the individuals which make up a species are widely different in these three groups of plants.

Lacking the precise information which would answer the question, what differences between species in these three genera may we logically expect on purely *a priori* assumptions, admitting for the purpose of the argument only that species are recognizable groups, the conclusion is unavoidable that they will, as units, be greatly affected by any factor which affects the degree of their isolation. Roughly we can classify these factors as internal and external. There must be many factors common to all Irises or to all Aquilegias which affect the nature and the degree of the isolation between species in each genus.

In the case of the genus Iris I think we may profitably consider not only that genus but practically the whole of the Liliiflorae (the Iris, Amaryllis and Lily families) since they are a natural group with many features in common. In the first place, polyploid series have been reported for a number of genera in these three families. That is to say that within many genera in this group (and sometimes even within the same species) forms occur whose chromosome numbers are simple multiple series. This will have a profound effect upon the relations between species.

To take a hypothetical example which differs in no essential detail from cases already reported in cytological literature, let XX and YY represent two diploid species, ZZŹŹ a tetraploid species, one letter being used to represent a haploid chromosome complement. If XX and YY, for instance, were species with twenty-four chromosomes (2n), ZZŹŹ would have forty-eight and each letter would represent a group of twelve chromosomes. XX and ZZŹŹ hybridizing produce a sterile

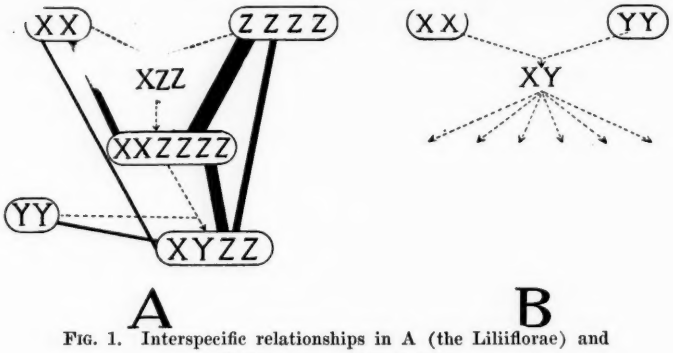

FIG. 1. Interspecific relationships in A (the Liliiflorae) and B (the genus Aquilegia).

triploid XZŹ, which by doubling gives rise to the hexaploid XXZZŹŹ. By analogy with such cases as are known we would expect the hexaploid to be fertile and true-breeding. These relationships are represented in Fig. 1, A, the broken lines indicating the genetic relationships of the species involved.

It is so commonly assumed in monographic work that morphological resemblance is a true guide to phylogenetic origin that it may be worth while to call attention to their differences in this particular example. The broad bands indicate the morphological resemblance to be expected on the assumption that it would be proportional to the number of sets of chromosomes in common. The example is a relatively simple one. As a matter of fact much more intricate interspecific relationships might very conceivably occur. Phylogenetic relationships and morphological resemblances in such groups will be reticular rather than dendritic. That is to say that the course of evolution, instead of being represented as a tree with diverging branches, may be more aptly likened to a complex and irregular web, with threads of varying thickness.

In the second place, the Liliiflorae are distinguished by highly developed vegetative propagation, as for instance in such genera as Tulipa, Lilium, Narcissus and Iris. Hybrids so sterile that in other groups of plants they

could not survive for more than a season or two may, by vegetative propagation, live for centuries and even spread across continents. There is therefore, among the Liliiflorae, the opportunity, on the one hand, for the production of complex, intergrading forms, and, on the other, for their survival and propagation. Newton and Darlington (1929) have shown that certain common tulips are sterile polyploid clones. Many of the bearded irises (Pogoniris) are sterile, and cytological examination proves them to be complex polyploids. Hybrids between *I. virginica* and *I. versicolor* are practically sterile, yet in certain localities, by vegetative propagation, they occur to the exclusion of the parent species.

In Aquilegia, on the other hand, there is little or no asexual propagation. It is difficult, even in an experimental garden, to keep a particular clone alive for more than four or five years. Sterile hybrids will therefore be of no importance. There is furthermore no polyploidy in the group, either among the wild species or the numerous garden forms which have been investigated. True-breeding hybrids can not therefore be produced, and only relatively homozygous hybrid segregates will perpetuate their kind.

Fig. 1, B, continuing the symbolism of the previous example, represents the results of a species cross in Aquilegia. The first generation hybrid, XY, being practically fully fertile, a flood of segregating forms will be produced in the second generation which will exhibit various recombinations of the characters of species XX and XY.

An actual case of such a cross between two species of Aquilegia is that of *A. formosa* of the Pacific Coast of North America and *A. flavescens* of the Rocky Mountains. Payson (1918) in his detailed monograph of the North American Aquilegias says:

> The greatest development of *flavescens* occurs in the higher mountains of Montana and adjacent Wyoming and Canada. As we go westward from this region we find the species apparently merging into *formosa;* the sepals become salmon-colored or pink, the laminae shorter, and the spurs straight.

This transitional area is often characterized by the lack of typical plants of either species, and in the mountains of Custer County, Idaho, the author has seen great patches of a variety with beautiful salmon-colored flowers entirely replacing the red *formosa* and the yellow *flavescens*. Since in the centers of their ranges *formosa* and *flavescens* are amply distinct, the author is very loath to treat one plant as a subspecies of the other. It would seem best to retain each as a species, never forgetting, however, that in certain regions the two actually merge.

There are furthermore in Aquilegia only slight physiological barriers to crosses between species, such as occur in practically all other genera. The most diverse Asiatic, American and European species cross readily and give fertile hybrids. Even *Aquilegia ecalcarata* Maxim. can be hybridized with *A. vulgaris,* though it is so distinct that Drummond and Hutchinson once (1920) placed it in Makino's genus Semiaquilegia. Species of Aquilegia will therefore be distinct only as long as that isolation persists. Whereas in Iris we may find several species growing together (as, for instance, *Iris versicolor* and *I. prismatica* in New England salt marshes) this will seldom or never occur in Aquilegia.

I have taken asexual propagation, polyploid series and physiological isolation as representatives of the internal factors which affect specific isolation and which whole genera or even families of plants may have in common. There must be many other such factors. May we not therefore logically expect that, even though species prove to be actual biological units, their relationships with each other and the relationships of individuals within species will vary from genus to genus and from family to family?

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SIZE GROUPS AND THEIR CHARACTERISTICS IN THE SALAMANDER *HEMIDACTYLIUM SCUTATUM* (SCHLEGEL)[1]

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IT has generally not proved possible to collect a large enough series of individuals of the four-toed salamander, *Hemidactylium scutatum* (Schlegel), for a clear definition of the age groups. Juveniles of the year, it is true, have been easy to recognize, and it is not difficult to get enough of these for a close approximation of the size limits at this age. The place to seek them is the dry, or damp, ground in the immediate vicinity of the nesting sites, as they do not wander far in the first autumn. The size limits of the adults, as well, may be fairly readily determined, although to get an adequate series of both sexes at the same time of year is not easy. The males may be had most easily in the autumn, for this is the mating season, and the females may be obtained in numbers at the nesting sites in the latter part of April and in May. Individuals intermediate in size between the yearlings and the adults may be found at all times of the year, but ordinarily very few at a time. The reasons for this are obvious. There are fewer individuals in this group than in the first-year group because there has been a year for their numbers to be thinned by such vicissitudes as enemies, accidents, weather, winter[2] and diseases; and the individuals are more scattered from their year of wanderings. Miscellaneous collections have given no evidence as to how many seasons this intermediate group represents.

[1] Contribution from the Zoological Laboratory of the University of Michigan.

[2] Mrs. Wilder has shown a heavy death-rate during the first winter for larvae of *Eurycea bislineata* (*Copeia*, 133: 78, 1924).

THE AGE GROUPS

One fortunate collection has now provided some real evidence on the question of age groups in this species. On the two days October 10 and 12, 1924, a series of 352 specimens of Hemidactylium was collected at a single locality in Hamburg township, Livingston County, Michigan, about fifteen miles north of Ann Arbor. In this collection the critical intermediate group was fairly well represented.

These specimens, as well as all others hereafter considered, were measured, freshly drowned, with dividers. For most comparisons the total length was found most useful, but the relative length of the tail has proved of value in characterizing certain groups as to age and sex. For this the tail length has been divided by the body length. The body length was taken arbitrarily as the distance from the tip of the snout to the posterior insertion of the hind leg, measured with dividers, and the tail was measured from this point to its tip. It was necessary, of course, to reject all specimens with incomplete or regenerated tails. The sex of juveniles was determined by dissection, but that of adults was often judged from the secondary sex characters.

Although we have collected Hemidactylium from more than twenty different places within a radius of twenty-five miles of Ann Arbor, Michigan, from only three has the species been obtained in numbers adequate to the present purpose. One of these places (Whiteoak, Ingham County) has furnished a fair series of adult females only; another (Iosco, Ingham County) has furnished only adults and yearlings, while from the other (Hamburg, Livingston County) has come the above-mentioned collection, the only one representing all ages in reasonable numbers in the same collection.

When the total lengths of the sexually immature males of this Hamburg collection are plotted by two-millimeter class intervals they form two well-defined and well-

separated groups (Fig. 1). The group containing the smallest individuals extends from 25 to 39 millimeters with a mode at about 29 to 33, and the group of larger individuals extends from 45 to 57 millimeters with an ill-defined mode at about 49 to 53 millimeters.

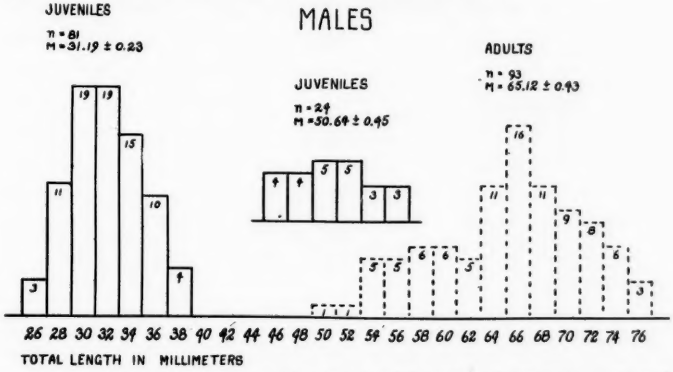

FIG. 1. Frequency polygons showing total lengths of all males collected at Hamburg, Michigan, October 10 and 12, 1924. The polygon of adult males is amplified by inclusion of 24 individuals collected at the same place on April 18 and 20, 1924. Specimens varying from 25.0 to 26.9 millimeters are plotted in the 26-millimeter group, etc. The number of specimens in each class is given near the top of each column.

The first group represents without question the individuals in their first season. The other group must be composed of individuals at least a year older. In the figure this group has been diagrammed on a short abscissa inserted above the principal one, in order not to interfere with the polygon of the adult group.

The polygon representing the distribution by total length of the sexually mature males of this collection overlaps notably that of the larger juveniles, with a conspicuous prominence well to the left of its mode at about 66 millimeters.[3]

[3] This polygon of adult males has been amplified by the measurements of 24 adults collected at the same place on April 18 and 20, 1924. There can be no objection to this inclusion because of the slow growth of adults and the very limited growing time between October 12 and April 20 in this locality. These added figures improve the bulk of the polygon without changing its shape.

It may now be asked whether the mature males shorter than 62 millimeters are of the same age as the larger juveniles, or are one year older. If of the same age, then we have an instance of a population of uniform size at the end of the first season diverging so in development during the next year that about half become sexually mature while the other half remain juvenile. Such divergence in rate of development within one age group is not unknown. Mrs. Wilder has shown[4] that in the salamander *Eurycea bislineata* great divergence in time of metamorphosis occurs during the second year of larval life. Transformation to the terrestrial form may take place at any time during the whole of the second season or even in the early part of the third season. Furthermore, Noble has recently pointed out[5] much variation in the time of attainment of sexual maturity in the newt *Triturus viridescens*. Hemidactylium, however, has a briefer egg-laying season than either Eurycea or Triturus and a shorter larval period than the former at least, so that its age groups may be expected to be more clearly defined.

If, in the present case, the larger juveniles and the smaller adults are of the same age they might be expected to show an approximately normal curve when plotted together. This they fail to do, but present on the other hand a low curve of greater width than would be expected for the second-year group.

While not denying that some individuals may become mature a season earlier than their class and that some may be delayed a season longer, the writers incline to interpret the figures as indicating that the smaller males in this species are a year older than the larger juveniles. In other words, the males ordinarily attain sexual maturity in southern Michigan at the end of their third season, *i.e.*, about two and a third years after hatching from the

[4] The Relation of Growth to Metamorphosis in *Eurycea bislineata* (Green). Journ. Exp. Zool., vol. 40, 1924, p. 2.

[5] Further Observations on the Life-History of the Newt, *Triturus viridescens*. Amer. Mus. Novitates, no. 348, 1929.

egg. Individuals of this age are assumed to be represented by the left-hand portion of the polygon of adults, and the modal point at 66 millimeters may well be chiefly due to males in their fourth, or a later, season.

The females collected on October 10 and 12, 1924, numbered 146, of which all but 12 were sexually immature, or non-breeding individuals. These 134 juveniles seemed clearly to belong to two size-groups (Fig. 2), separated by a well-marked interval at about 44 millimeters of total length. The group of smaller individuals is unquestionably a unit. It shows a mode at 31 to 33 millimeters and an extreme variation from 27 to 43 millimeters. The

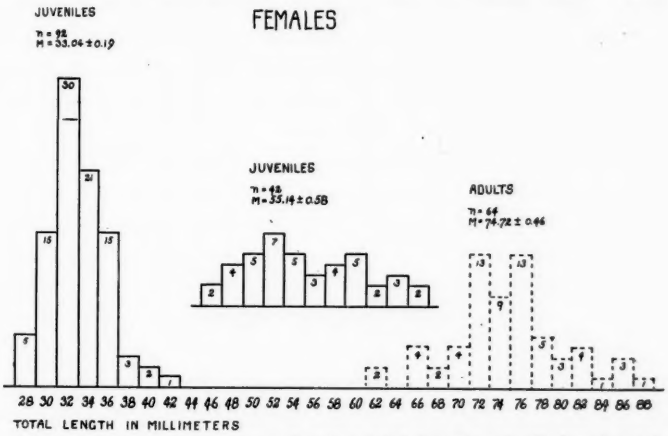

FIG. 2. Frequency polygons showing total lengths of all females collected at Hamburg, Michigan, October 10 and 12, 1924. The polygon of adult females is amplified by inclusion of 52 individuals collected at the same place on April 18 and 20, 1924. The number of specimens in each class is given at the top of each column. Specimens varying from 27.0 to 28.9 millimeters are plotted in the 28-millimeter group, etc.

other group of juveniles, composed of only half as many individuals, seems to be a compact group although it shows no clear mode. Its range of variation, 45 to 67 millimeters, is, as would be expected in an older population, a little greater than that of the younger juvenile group. One specimen 73 millimeters long, although a

non-breeder, is not included in this group, for an unusually heavy infestation of the protozoan parasite *Haptophrya michiganensis*[6] led to the inference that this was an adult which had been unable to produce eggs.

Since the series of twelve sexually mature females in this collection is too small to show the position of the adult group with respect to the larger juveniles, we have added the measurements obtained from a lot of 52 adult females collected at the same place, on April 18 and 20, 1924. On account of the relatively insignificant growing season between October 12 and April 20 in this latitude and the fact that none of these 52 individuals had laid their eggs, there can be no objection to using these April specimens for determining the range in size of the breeding females.[8] The adult group thus shown (Fig. 2) overlaps slightly the group of larger juveniles, as was noted to be the case with the male sex.

Further evidence bearing on the question of the unity of age of the intermediate group may be found in the relative length of the tail. Such evidence is more particularly to be desired for the females because the polygon formed from the total lengths of the intermediate sizes in this sex is evidently bimodal (Fig. 2). When the relative tail length of these individuals is plotted into a frequency polygon at class intervals of 0.05 a unimodal polygon of sufficiently normal form is obtained. We may from this feel safer in our interpretation that the juveniles intermediate between the yearlings and the adults belong to a single age group. A comparison of this polygon with that for the yearlings and adults is given in Fig. 3.

The frequency polygon for the male sex, based on the relative tail length (Fig. 4), looks much like that based on total length (Fig. 1), except that, as in females, there is considerable overlapping between the smaller adults and the larger juveniles.

[6] Woodhead, A. E., *Haptophyra michiganensis* sp. nov. Journ. Parasit., vol. 14, 1928, pp. 177–182.

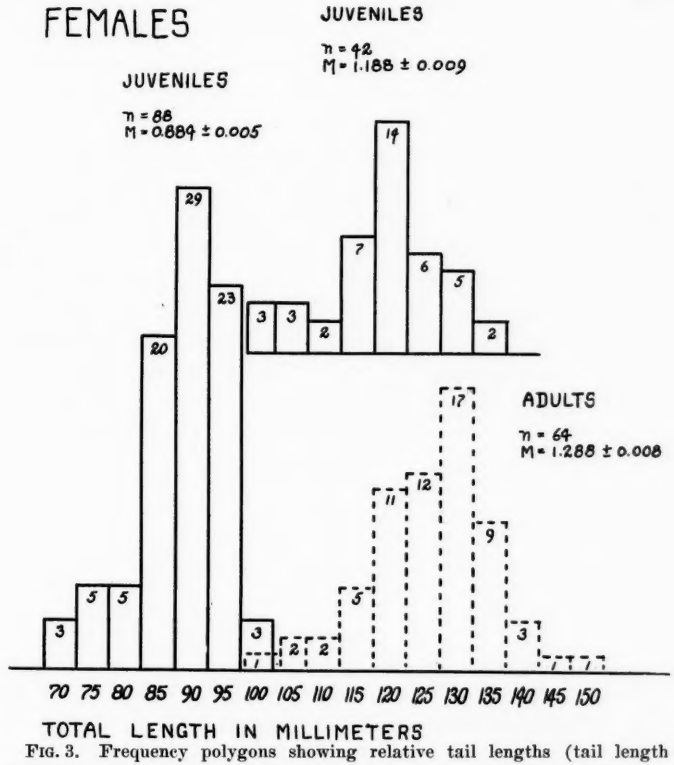

FIG. 3. Frequency polygons showing relative tail lengths (tail length divided by body length) of all females collected at Hamburg, Michigan, October 10 and 12, and, adults only, April 18 and 20, 1924. The number of individuals in each class is given at the top of each column. Specimens varying from 0.675 to 0.724 are plotted in the 0.70 class, etc.

An attempt to get direct evidence of the size of the different ages of these salamanders provided data that, so far as they go, are supportive of the conclusions derived from the measurements discussed above. A cement enclosure about 8 x 10 feet was made in a shady place and provided with a pond and woods earth and logs. Eggs from natural nests were put in moss appropriately situated above the water, in the spring of 1923. On October 23 eleven small individuals with complete tails

were recovered from the enclosure. These averaged 31.85 millimeters in total length, with a range of variation from 30.0 to 35.5 millimeters. This checks perfectly with the yearlings collected in the field at this time of year (Table II, Figs. 1 and 2).

These salamanders were revived (having been anesthetized with water for the measuring) and returned to the enclosure for another year's growth. In the spring of 1924 more eggs were put in the pond to provide a new crop of yearling salamanders for the coming autumn.

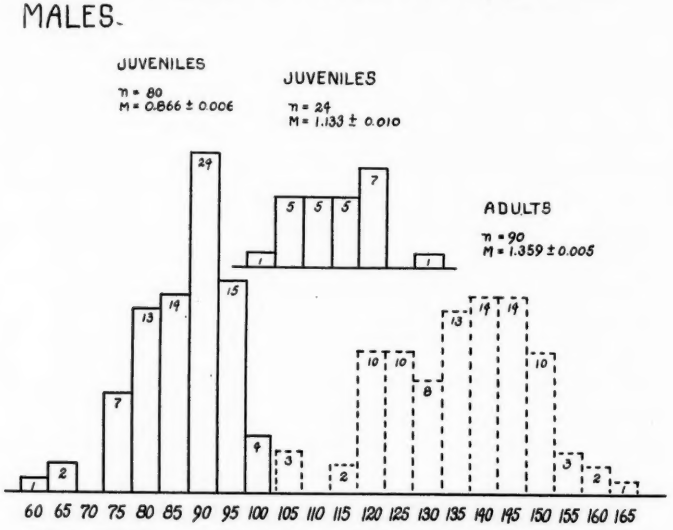

FIG. 4. Frequency polygon showing relative tail lengths (tail length divided by body length) of all males collected at Hamburg, Michigan, October 10 and 12, and, adults only, April 18 and 20, 1924. The number of individuals in each class is shown at the top of each column. Specimens varying from 0.725 to 0.774 are plotted in the 0.75 class, etc.

On October 22, 1924, the salamanders recovered from the enclosure were clearly of two size-groups, but they were all smaller than expected (Table 1). This may probably be attributed to less favorable conditions (apparently dryness), since during the ensuing season all the

salamanders disappeared from the enclosure. These two groups may, however, fairly be said to represent the two groups of juveniles that may be collected from natural habitats at this time of year. The difference between the mean total lengths (16.77 millimeters) of these two groups is only a little less than the difference between the means of the two groups of males (19.44 millimeters) collected at Hamburg at the same season of that year. In the corresponding differences between the means of the proportionate tail lengths the salamanders from the enclosure made the greater gain (0.316 in comparison with 0.265). Since a greater relative increase was made in tail length it is, perhaps, reasonable to assume that there was insufficient natural food in the enclosure to provide for normal increase in the bulkiest part of the animal, *i.e.*, the body.

TABLE I

JUVENILE SALAMANDERS COLLECTED OCTOBER 22, 1924, FROM ENCLOSURE

	Total length in millimeters	Tail divided by total length
Group of smaller juveniles	26.0	0.852
	26.5	0.783
	27.1	0.807
	28.7	0.817
	29.0	0.791
	29.3	0.773
	29.8	0.807
	M = 28.06 ∓ 0.32	0.804 ∓ 0.006
Group of larger juveniles	42.7	1.128
	43.0	1.128
	44.0	1.061
	45.2	1.148
	46.2	1.162
	47.9	1.095
	M = 44.83 ± 0.52	1.120 ± 0.009

CHARACTERISTICS OF THE AGE GROUPS

These salamanders in their first autumn can be recognized as yearlings by their small size alone. The largest

individual in the two principal collections made (totaling 334 specimens) was 41.6 millimeters in length, and the averages of the four groups were from 30 to 33 millimeters (Tables II and III). Between the two sexes at this age there is certainly no obvious difference in length,

TABLE II

COMPARISON OF THE SEXES AT DIFFERENT AGES IN A COLLECTION OF HEMIDACTYLIUM MADE AT HAMBURG, MICHIGAN, ON OCTOBER 10, 12 AND (ADULTS ONLY) APRIL 18, 20, 1924. MEASUREMENTS ARE IN MILLIMETERS

Age	Sex	No.	Variation	Mean	Difference between means	Difference divided by probable error
			Total length			
First autumn	males	81	25.8 –38.3	31.19 ± 0.23		
"	females	92	25.8 –41.1	33.04 ± 0.19	1.85 ± 0.30	6.2
Second autumn	males	24	45.3 –56.8	50.64 ± 0.45		
"	females	42	45.5 –66.9	55.14 ± 0.58	4.50 ± 0.63	7.1
Adult	males	93	49.8 –76.6	65.12 ± 0.43		
"	females	64	62.0 –87.7	74.72 ± 0.46	9.60 ± 0.63	15.2
			Tail length divided by body length			
First autumn	males	80	0.612– 1.016	0.866 ± 0.006		
"	females	88	0.678– 1.030	0.884 ± 0.005	0.018 ± 0.008	2.3
Second autumn	males	24	1.012– 1.290	1.133 ± 0.010		
"	females	42	1.000– 1.366	1.188 ± 0.009	0.055 ± 0.014	3.9
Adult	males	90	1.050– 1.648	1.359 ± 0.005		
"	females	64	1.029– 1.484	1.288 ± 0.008	0.071 ± 0.009	7.9

but it is noteworthy that the females in each of the collections, Iosco and Hamburg, showed a little higher average total length than the males. In the Iosco collection this was a little over one millimeter and in the Hamburg collection nearly two millimeters. In the former case the difference in length between the sexes exceeded the probable error of the difference by three times and in the latter by a little more than six times. These small differences might not be regarded as significant if it were not for the fact that they are greatly accentuated in the adults, so it is quite possible that the females may be growing faster than the males even at this early age.

TABLE III

COMPARISON OF THE SEXES AT DIFFERENT AGES IN A COLLECTION OF HEMIDACTYLIUM MADE AT IOSCO, MICHIGAN, ON NOVEMBER 9, 1924, AND (ADULTS ONLY) NOVEMBER 16, 1924, APRIL 5 AND NOVEMBER 22, 1925, AND OCTOBER 17 AND 31, 1926. MEASUREMENTS ARE IN MILLIMETERS

Age	Sex	No.	Variation	Mean	Difference between means	Difference divided by probable error
			Total length			
First autumn	males	76	22.1 –37.8	30.34 ± 0.29		
"	females	85	19.9 –41.6	31.55 ± 0.26	1.21 ± 0.41	3.0
Adult	males	83	60.2 –87.9	73.98 ± 0.41		
"	females	41	69.7 –94.7	83.66 ± 0.54	9.68 ± 0.68	14.2
			Tail length divided by body length			
First autumn	males	76	0.675– 1.000	0.828 ± 0.006		
"	females	86	0.566– 1.071	0.831 ± 0.007	0.003 ± 0.009	0.3
Adult	males	81	1.173– 1.844	1.490 ± 0.009		
"	females	42	1.173– 1.600	1.377 ± 0.011	0.113 ± 0.014	8.1

Another feature of the yearling group is the notably short tail, a character that can be readily appreciated with the specimens in hand. In salamanders of this age the tail is a little shorter than the body, while in those a year or more older it is nearly always a little longer, unless injured or regenerated. Regenerated tails can usually be recognized by their slightly different appearance from normal tails. They are apt to be a little shorter and thicker and to have less sharply defined black markings on the under side. The mean tail length divided by the body length varied in the two collections of yearlings from 0.83 to 0.88. Although these figures were slightly higher in the females in each case, this difference may be regarded as insignificant from the fact that it exceeded the probable error of the difference less than three times (0.3 and 2.3), and also from the fact that in adults this proportion is markedly greater in the other (male) sex.

Individuals comprising the group intermediate between the yearlings and the adults (and here regarded as

at the end of their second season of life) are separated from the yearlings on total length measurements by a distinct gap, but overlap distinctly with the smaller adults. Only one collection was obtained in which this group was at all adequately represented. In this the mean of 24 males was 50.6 millimeters and of 42 females 55.1 millimeters. The difference, which is further accentuated in the adults, exceeded its probable error about seven times (Table II).

The tail length divided by the body length in the salamanders at the end of the second season varies from 1.00 to 1.37, with means of 1.13 and 1.19 for the males and females, respectively. The slight difference between the sexes exceeds its probable error by 3.9 times. This difference may be regarded as of doubtful significance since in adults it is the males that have the longer tails.

As in the yearling group, individuals of the second season are not externally distinguishable as to sex.

The adults are readily recognizable as such in autumn and early spring, but after the eggs are laid the smaller females may not be distinguishable from the larger juveniles. The measurements on which the following comparisons are based were taken from adults collected at various dates from October 10 to the end of the season (usually about one more month in this vicinity) and in early April, before the eggs were laid. The collections were made at Hamburg and Iosco. The variation in the total length of the males was from 50 to 88 millimeters with averages of 74 and 65 in the two collections (Tables II and III). In the females there was variation from 62 to 95 millimeters, with averages of about 84 and 75 in the two lots (Tables II and III). The average difference between the sexes was the same in each case. The females were 9½ millimeters longer than the males (Tables II and III).

The relative length of the tail in the adults, although on the average distinctly greater than in the two-year-old individuals, practically wholly overlaps the figures for

this latter group. The variation is from 1.03 to 1.84. The averages are from 1.28 to 1.49. The difference between the sexes was 0.07 in one collection and 0.11 in the other, differences that exceed their probable errors by about eight times (Tables II and III).

The adult male may be recognized by its more truncate snout with larger swellings in the regions of the naso-

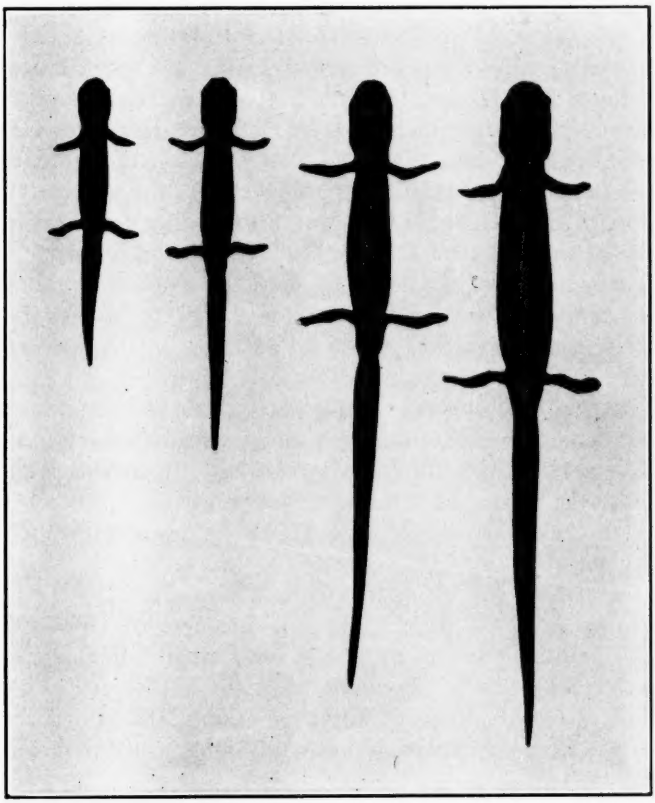

FIG. 5. Photograph of individuals of *Hemidactylium scutatum* representing the three size groups in autumn. Left to right: juvenile in its first autumn, juvenile in its second autumn, adult male, adult female.

labial grooves and by the smaller amount of pigment in this region than in the female, as well as by its shorter and more slender body and longer tail.

The relative sizes and proportions of individuals representing the three groups are shown in Fig. 5. The two salamanders at the left are juveniles of the first and second autumns, respectively; the two at the right are adult male and female individuals, respectively.

VARIATION OF SIZE WITH HABITAT

Unexpectedly it was observed during the compilation of these figures that the adults from the Iosco habitat were decidedly larger than those from the Hamburg collecting place. The males as well as the females from Iosco averaged 9 millimeters longer than the respective sexes from Hamburg, differences exceeding their probable errors by 12 to 15 times (Table IV). The proportionate length of the tail varied in the same way. The differences between the means for the two localities exceeded their probable errors by 6½ times in the females and 13 times in the males.

Although the young salamanders in their first season showed differences, in these measurements, between the same sex in the different places, these differences were relatively slight, and furthermore, they were reversed, *i.e.*, the salamanders from the Hamburg locality were the larger.

One other collection, that of 46 adult females from Whiteoak, has a bearing on this problem of variation with habitat. The mean of the total lengths in this collection was practically identical with that of the adult females from Hamburg, differing by only 0.6 of a millimeter; and the proportionate tail length differed but slightly more.

These figures are too few to prove anything but they suggest the possibility of significant variation between habitats. It would be mere speculation to try to account for the marked similarity between the adult females from

TABLE IV

COMPARISON OF LIKE SEXES AND AGES OF HEMIDACTYLIUM FROM DIFFERENT HABITATS

Place	Age	Sex	No.	Mean	Difference between means	Difference divided by probable error
				Total length		
Hamburg	1st autumn	male	81	31.19 ± 0.23		
Iosco	"	"	76	30.34 ± 0.29	0.85 ± 0.30	2.8
Hamburg	1st autumn	female	92	33.04 ± 0.19		
Iosco	"	"	85	31.55 ± 0.26	1.49 ± 0.32	4.7
Hamburg	adult	male	93	65.12 ± 0.43		
Iosco	"	"	83	73.98 ± 0.41	8.86 ± 0.59	15.0
Whiteoak	adult	female	46	74.09 ± 0.62		
Hamburg	"	"	64	74.72 ± 0.46	0.63 ± 0.77	0.8
Hamburg	"	"	64	74.72 ± 0.46		
Iosco	"	"	41	83.66 ± 0.54	8.94 ± 0.71	12.6
				Tail length divided by body length		
Hamburg	1st autumn	male	80	0.866 ± 0.006		
Iosco	"	"	76	0.828 ± 0.006	0.038 ± 0.0085	4.5
Hamburg	1st autumn	female	88	0.884 ± 0.005		
Iosco	"	"	86	0.831 ± 0.007	0.053 ± 0.0086	6.2
Hamburg	adult	male	90	1.359 ± 0.005		
Iosco	"	"	81	1.490 ± 0.009	0.131 ± 0.010	13.1
Whiteoak	adult	female	44	1.251 ± 0.011		
Hamburg	"	"	64	1.288 ± 0.008	0.037 ± 0.014	2.6
Hamburg	"	"	64	1.288 ± 0.008		
Iosco	"	"	42	1.377 ± 0.011	0.089 ± 0.014	6.4

Whiteoak and Hamburg and their marked difference from those of Iosco. We will merely observe, as having a possible bearing, that the Hamburg and Whiteoak localities are relatively limited and isolated and that the Iosco place is part of an extensive swamp.

SUMMARY

1. Evidence has been presented in favor of the view that Hemidactylium attains sexual maturity near the end of its third season of life, *i.e.*, when it is approximately two and a third years old.

2. Juveniles in their first autumn can be recognized by their small size (about 26 to 41 millimeters of total length) and by their short tails (less than the length of the body measured to the posterior insertion of the hind leg). There is practically no difference in length between the sexes at this age, but the mean for the females is slightly, perhaps significantly, greater than that for the males.

3. Juveniles in their second autumn are distinctly longer than the yearlings, but as a group they overlap in length the shorter portion of the adult group. The females average about four millimeters longer than the males. The relative length of the tail is not significantly different between the sexes at this age. It is distinctly different, however, from that of the yearlings, for it is nearly always longer than the body.

4. The adults are, of course, larger than the juveniles, except for some overlapping. The females are longer by 9½ millimeters, on the average, than the males, and, when bearing eggs, have distinctly wider abdomens. The males have relatively longer tails than the females, and are recognizable otherwise by differences in the shape of the head.

5. There is some evidence that differences in local conditions may have a decided influence on the sizes attained by the different populations.

HYBRID VIGOR—A FACTOR IN TETTIGID PARTHENOGENESIS?[1]

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IT has been shown by Nabours (1919, 1925, 1929), Nabours and Snyder (1928) and Nabours and Foster (1929) that heterozygous partheno-reproducing Tettigidae give segregates, practically all females, that are with rare exceptions homozygous. Furthermore, such females when allowed to mate produce offspring that show the characters of both parents as well as some that show only matroclinous characters (*loc. cit.*). Robertson (1930*a*) has shown that partheno-produced individuals are diploid and also that in their somatic and gonial cells like chromosomes have a tendency to lie together. The discovery of Nabours and observations of Robertson suggest that the partheno-produced tettigid arises from an egg that has given off but one polar body. Also, the breeding results (Nabours, 1919, etc.) show that the mechanism must be one that provides simultaneously for parthenogenetic and biparental reproduction. It may be supposed that the egg normally gives off the first polar body and waits for the sperm to enter. In the absence of the sperm, after some delay, the eggs of most females may degenerate, but eggs of some females evidently have the capacity to proceed with development without the second maturation having taken place.

That apogamy and parthenogenesis are in some way connected with hybridization was first suggested by Ernst (1917, 1918), as a result of his studies on the stoneworts. He noted that in varieties exhibiting such methods of reproduction the vigor and general character of the plants, the numbers of chromosomes and their irregu-

[1] Contribution No. 122, department of zoology, Kansas Agricultural Experiment Station, Manhattan.

larities during the meiotic divisions and the absence of such irregularities in bisexual varieties were suggestive of hybrids. These conclusions were supported by a number of other plant workers (Rosenberg, 1917; Winge, 1917; Holmgren, 1919; Täckholm, 1920, 1922; *et al.*). Harrison and Peacock (1925) in their studies on moth crosses came to the conclusion that hybridity was a factor in bringing about parthenogenesis. They mentioned heterosis but did not emphasize it. When the work of Nabours (1919, 1925) on *Apotettix* came to their notice they saw that he had brought together colonies from different regions and suggested that in the Tettigidae also there might be a "case of parthenogenesis consequent upon hybridity" (Peacock and Harrison, 1926). Nabours (1929) and Nabours and Foster (1929) accepted this as a possible cause but added the fertile suggestion that parthenogenesis might have come about through the bringing together of complementary factors that had previously been segregated in regional varieties.

Whatever the cause of hybrid vigor may ultimately be found to be, whether a matter of stimulation, a presence of a combination of dominant size factors, heterozygosis itself as such, a series of linked or unlinked growth factors, or in some cases homozygous hybridity (recombinational from species' crosses), it would seem that all theories should agree in this: that it is brought about by hybridization, and that it may express or manifest itself in such ways as rapidity of growth, increased ultimate size, increased fecundity and the like. These phenomena in turn involve increase in size of the cell and with it increased rate of cell division. Hybrid vigor may therefore be looked upon possibly as having a speeding-up effect on cell division and of course among other things upon the growth and resultant division of the chromosomes.

It seems reasonable then to suppose that hybrid vigor might be expressed in the oocyte and egg of these tettigid hybrids in such a way as to advance the growth and con-

sequent splitting processes in the chromatin threads during synapsis and succeeding stages to the extent that after the first polar body, *e.g.*, is thrown off the chromatids of the diads may each have received an impetus sufficient to cause them to continue these processes during the supposed period of the pause for the entrance of the sperm. As a result of this they may be supposed to have proceeded far enough in their division to form the first cleavage nucleus, in this way starting the development of the embryo. Hybrid vigor, through advancing the condition of the chromosomes, might therefore possibly serve as the stimulus to push the delaying egg over into parthenogenetic development.

However, if hybrid vigor be accepted as a factor bringing about parthenogenesis in the Tettigidae, and it be assumed that this vigor is due to heterozygosity, the following fact looms in opposition. The F_1 individual which has the capacity for parthenogenesis is a hybrid, but Nabours (1925, 1929) has found the F_2 (with rare exceptions) and succeeding parthenogenetic generations to be homozygous for their characters and yet parthenogenesis to continue. Obviously the hybrid vigor push should be absent in the eggs of F_2 and later generation females unless the vigor stimulus be some substance in the cytoplasm that is handed on in gradually diminishing amount. But there is a possibility that the F_2 and later generation parthenotes[2] may not be homozygous for all their characters. As has been shown by Robertson (1930*a*) one maturation division, and that reductional in the main, would take care of segregation such as is shown by the breeding of heterozygous parthenoreproducing females, and the equational division if omitted or if it becomes the first cleavage mitosis would agree with the occurrence of homozygosity in the segre-

[2] The term *parthenote* is here proposed for the designation of an individual that has developed from an egg containing a single pronucleus, and should correspond in its usage to the term *zygote* which designates an organism that has arisen from an egg in which there were two pronuclei.

gates and their descendants. It has been known for some time that in the Tettigidae homologous chromosomes are split before they pair in parasynapsis (Robertson, 1916, 1917, 1919, 1930*b*). The same had been discovered by Bridges (1916) in Drosophila from genetic data. If this be well established for the Tettigidae then it would be expected that, in addition to four-strand crossovers at one locus, there should occasionally be simultaneously a two-strand crossover at a different locus, thus resulting in four different strands to be distributed at the two maturation divisions. If this be the case, that it may be suspected will be shown later, when more distantly linked genes are discovered (*e.g.*, the θ character in item 41, Table II, and mating 229, Table V, in Nabours and Foster [1929] for Paratettix), one maturation division would not suffice. In *Apotettix* among the twelve different factors reported for five different loci (Nabours, 1925) the highest average crossing-over percentage between the genes at the extreme ends of the linkage group is 7.43. This would indicate a rather close linkage and probably a very small proportion of the length of a chromosome to be involved. If such be shown to be the case, and it be accepted as proved cytologically that the second (so-called equational) division is omitted in parthenogenesis (Robertson, 1930*a*), it would be expected that some of the F_2 segregates should exhibit, with respect to some of their characters, either a uniform hybrid condition, or a mosaic condition (two types of homozygous parts) with reference to the right and left or anterior and posterior portions of the body. This would depend of course upon whether the first cleavage mitosis occurs along the split between chromatids, that should have taken place at the second maturation, or whether it occurs along the real equational split in each chromatid itself. In one case the soma would exhibit a uniform heterozygous condition and the germ-cells would show both segregation and crossing over. In the other case the soma might be ex-

pected to exhibit, so far as this particular character is concerned, mosaics, and the germ-cells might be of two sorts (each homozygous) exhibiting cellular segregation but no crossing-over phenomena.

From the cytological evidence therefore and from the fact that all the factors so far considered are in such a short linkage group the conclusion would not be warranted that the F_2 parthenogenetic segregates should be homozygous for all their genetic factors. They might be so for the short linkage group which was being dealt with in *Apotettix* (Nabours, 1925), but for those factors that should have been segregated in the second maturation division, that evidently did not take place, they should be heterozygous. If both maturation divisions are concerned in segregation, possibly there might be differing amounts of segregation in each division, the first effecting the major part of it, the second the minor part. F_2 parthenotes should then for the majority of their genes be homozygous segregates, but to a less extent for other characters they should be heterozygous. Through succeeding parthenogenetic generations the offspring should gradually become homozygous for all their characters. If hybrid vigor is bound up with heterozygosity then parthenogenesis, in case it be induced by hybrid vigor, should gradually disappear. On the other hand, if parthenogenesis be due to the bringing together of complementary factors (Nabours, 1929) a basis would be afforded for a permanent (homozygous) condition of heterosis (hybrid vigor), and with it the possibility of continued parthenogenesis. The breeding results should decide between these suggestions, and possibly throw light upon the cause of hybrid vigor itself. First of all it should be established cytologically that but one polar body is thrown off (we are fairly certain of this now [Robertson, 1930*a*]). In the second place it should be established genetically whether the F_2 and later generations of parthenotes are totally homozygous (the distantly linked gene, θ, in *Paratettix* seems

to indicate they are not [Nabours and Foster, 1929]), or in part heterozygous, and in the third place whether parthenogenesis in the Tettigidae may be continued indefinitely (it has been continued for seven generations in *Apotettix* [Nabours, 1929]).

The hybrid vigor hypothesis might be applied to some cases of alternation of parthenogenetic with biparental reproduction such as occurs in rotifers, aphids and *Hymenoptera.* Whitney (1912) and Shull (1912) found in rotifers that continued parthenogenesis resulted in loss of vigor, finally ending in the production of a bisexual generation which in turn started anew the parthenogenetic portion of the cycle. In the Tettigidae parthenogenesis and alternation seem to be in an incipient condition. In the rotifers and the aphids both types of reproduction and their alternation are well established, but the parthenogenetic portion of the cycle seems to be indefinite and capable of being prolonged. This seems to argue against hybrid vigor of the heterozygosity type as being a factor bringing about parthenogenesis, although it has been established that the second polar division does not take place (Whitney, 1909), and of course the possibility remains that the parthenogenetic lines may in the early generations at least be heterozygous. In the majority of the *Hymenoptera* the alternation seems fixed and is limited to one bisexual generation alternating with one parthenogenetic generation. Is it possible that hybrid vigor, as a cause of parthenogenesis, induced by wide crosses in the Tettigidae has become incorporated in ordinary bisexual reproduction within the variety in (the rotifers and) *Hymenoptera?* Are we certain that the male in the bee is haploid? Is it possible that he might be diploid and the female tetraploid? The "coupling" (Nachtsheim, 1913) of like chromosomes in the second spermatocytes and somatic cells of the male and in the oogonial and somatic cells of the female may in some way be a case of the association

of homologues similar to that which occurs in the partheno-produced Tettigidae (Robertson, 1930*a*). Is there a possibility of combining these suggestions with that of Nabours' (1929) complementary factor hypothesis (which essentially means homozygous for heterosis-producing factors) in an explanation of the alternation of biparental with parthenogenetic reproduction as it occurs in this group?

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SEX DIMORPHISM AND SCHOOLING BEHAVIOR AMONG FISHES

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IN a previous paper[1] the writer has attempted to show how the psychological mechanism involved in the aggregation and maneuverings of individual fishes in schools may probably be explained as composed of a very simple set of purely automatic reactions. Greater difficulties are met with in an endeavor to explain the phylogenetic or ontogenetic origin of these reactions. It is not *a priori* inconceivable that they should properly be regarded as manifestations of ontogenetically acquired habits, but their early appearance in the life of the individuals and their more or less fixed relationship to definite systematic groups of fishes indicate the probability of their being based upon the phylogenetically developed, inheritable, nervous and morphological organization of the species after the manner of tropisms and sex instincts.

With our present inadequate knowledge of the physical background for instincts and nervous reactions, it is impossible to attempt any direct explanation for the evolution of the schooling "complex," unless the theory of phylogenetic adaptation according to the requirements of the organism be dogmatically adopted as a necessary axiom of biology. But, granting the evolution of the schooling instinct as an inexplicable quality in the nervous organization of many fishes, we may be able to determine some of the factors influencing its varying degrees of manifestation in the different systematic or biological groups, by studying the relation-

[1] A. E. Parr, "A Contribution to the Theoretical Analysis of the Schooling Behavior of Fishes," Occasional Papers, Bingham Oceanographic Coll. No. 1, 1927.

ship of the schooling behavior to the entire habit patterns of the various forms.

In the case of the inherent reactions of any organism the simple elements must be considered in their coordination with each other and their subordination to the whole to a far greater extent than in the study of the merely structural morphology, as almost any one of the many instincts centered around the various stimulations to which the organism is able to respond may temporarily or permanently serve to suppress the manifestations of any or all of the other complexes in the nervous organization of the species, or may equally serve to increase their external effect. It will be sufficient to mention the complete suppression of even such a fundamental psychological factor as the feeding instinct during the breeding season of many fishes. Without in any way intending to attribute to the fishes a capacity for consciously harboring complexes of "suppressed desires," the writer simply wants to call attention to the fact that certain normal, even vital, inherent reactions may partly or completely disappear for a considerable length of time during the life of the individual fish, while the neurophysiological mechanism of the instinct involved apparently remains intact and ready to respond as soon as the inhibiting complex returns to a passive state. In discussing the occurrence and varying development of the schooling behavior of the different forms we must therefore not only observe the external manifestations of the schooling instinct, but we must also consider the possibility of these manifestations having been altered, promoted, reduced or even entirely suppressed by the interference of other compatible or incompatible instincts in the psychological make-up of the species considered. Our task is, then, to try to make out the possible inhibiting or promoting factors in the established habit-patterns of recent fishes, thereby trying to find an explanation for the occurrence and development of their

schooling performances as such, without considering the origin of their schooling instinct in itself.

Among the factors which may possibly be of importance in this respect it is natural first to consider the sexual complexes, as these are the only, or at least by far the most important, elements in the nervous organization agreeing with the schooling complex in having for their adequate stimulus the perception of other individuals of the same species. A comparatively strong interference by the corresponding responses should therefore be expected to develop.

In the ideal school, which is very closely approached by the actual schools of such forms as the herrings or mackerels, the individual fishes are equidistantly adjusted to parallel directions of swimming, according to their mutual attraction for each other, in the manner already discussed and explained by the author (*loc. cit.*). In a school of this kind the sexes are freely mixed without any differentiation in their behavior or spatial arrangement. If now a differential sex attraction develops, it is obvious that the uniform aggregation of schools will no longer be insured by a homogenous stimulation of all individuals in a given population. Points of weakness will develop between fishes of the same sex and points of comparatively strong attraction between opposite sexes. Uniform schooling can not even under these circumstances be regarded as *a priori* impossible, but it is practically inconceivable that schools of any considerable size or uniformity should be able to aggregate and remain together under such conditions, while exposed to the constantly disturbing and disrupting influences of their natural environment. Any aggregation of individuals would rapidly break up into smaller units or pairs of a heterosexual nature. But, for a differential sex attraction to develop, it is necessary that there should be a perceptible difference between the sexes. Very little is known about the adequate stimuli for the sexual attraction among fishes, but it is logical to assume that

they should in most cases be of the same sensory quality as the stimuli for the analogous, but asexual, schooling attraction. The writer has already previously (*loc. cit.*) by experiment and general considerations attempted to show that the common and most perfectly harmonious type of schooling performances is probably entirely based upon visual perceptions alone, and we will therefore in the following discussions assume the visual perceptions to be also, in general, the causes of sexual attraction among fishes, testing the justification of our assumption by the validity of the conclusions it will lead us to. Our theoretical condition for a differential sex attraction to develop is, then, that there must be an externally visible difference between the sexes. If such differences are not present, the reacting individual is without means of discriminating the opposite sex from its own, and consequently can not be more strongly stimulated by one than by the other. What peculiarities the nervous organization of a species may develop under such circumstances is a matter of conjecture, but it seems obvious to the writer that the mechanism of sex attraction, if not entirely replaced by an already developed schooling instinct, can not become lost without leading to the immediate extinction of the species for lack of adequate propagation. It is rather to be expected that the mechanism must, in the surviving forms, have changed from heterosexual to an ambisexual orientation, causing attraction towards all individuals of the same species irrespective of their sex. The sexual complex itself may in other words be supposed to have changed into an instinct for indiscriminate schooling behavior.

Comparing these theoretical conclusions with the actual observations we find that the groups showing the most perfectly harmonious schooling performances, such as herrings and mackerels, are conspicuously lacking in external sex differentiation, and also do their spawning in indiscriminate schools, apparently in most cases without any indication of a heterosexual orientation. Even

the expert with years of experience in the study of the common *Clupea harengus* is unable to determine its sex without examining its gonads or genital products. Our knowledge about the occurrence of sex dimorphism among fishes is unfortunately rather scanty, but where adequate information is available, we find as a general rule that schooling behavior is not observed in conjunction with conspicuous external sex differentiation. The case is particularly clearly illustrated by the fauna of the tropical coral reefs where the adult representatives of the strikingly dimorphous families *Labridae* and *Scaridae* have never been observed by the writer to exhibit any indication of a schooling behavior, while the monomorphous *Haemulidae* are usually moving around in the same environment in indiscriminate schools of varying sizes. Similar evidence is contributed by the fact that schooling performances are far more commonly found among the immature than among the mature fishes.

The list of indiscriminately and more perfectly schooling types, such as the herrings and mackerels, can, on the other hand, be increased by numerous species among the less conspicuously differentiated or absolutely monomorphous forms of such families as the *Gadidae, Atherinidae, Mugilidae, Belonidae, Scomberesocidae, Hemiramphidae, Exocoetidae, Carangidae* and *Gerridae,* and by several *Cyprinidae, Salmonidae* and *Sciaenidae.*

Even more interesting evidence is contributed by the only periodically dimorphous forms, such as the common European stickleback, *Gasterostheus aculeatus,* whose males, during the breeding season, develop a very brilliant red color on the otherwise silvery belly. Concomitantly with the appearance of this character, the rather perfect schools of the species in question rapidly disintegrate and the social and indiscriminate schooling instinct gives way to a very pronounced and pugnacious individualism with a heterosexual orientation towards the former companions. After the spawning season is

over the bright coloration disappears and indiscriminate and very harmonious schools are again observed.[2]

Parallel conditions also seem indicated in the seasonal differences in the schooling behavior of various freshwater fishes observed by Evermann and Clark[3] and particularly clearly described in the case of *Pimephales notatus* concerning which these authors report (*loc. cit.*, p. 343) that the specimens are usually found "singly or a few together" during the spring and summer, "but later they bunch up and in the fall and winter they are found in considerable schools," the scattering thus centering around the breeding season of the species (given as June). Similar observations are also recorded for *Notropis blennius* (*loc. cit.*, p. 351) and for *Fundulus diaphanus* (p. 371). In their breeding habits these species, like the stickleback, show a temporary ability to react heterosexually.

Evidence of a similar correlation between sex attraction and schooling behavior in perfectly monomorphous forms may possibly also be borrowed from A. C. Johansen's[4] explanation of the regular, annual disappearances staged by most of the European races of herring (*Clupea harengus*) as being due to an extensive scattering mainly of the adult fish, when, "as spents or recovering spents, they go hunting for food in a more pronounced degree than otherwise." While there is obvious reason to believe, with Johansen, that the feeding instinct would

[2] This description of the seasonal changes in the social orientation of *Gasterosteus aculeatus* is based upon many years' observation of this species in European waters. The fact that the stickleback, outside of its spawning season, is found in typical, indiscriminate schools is only occasionally referred to in the literature (*vide* C. V. Otterström, "Fisk. I. Pigfinnelisk," p. 114–115, in "Danmarks Fauna," Copenhagen, 1912), most authors being too preoccupied with the fascinating breeding habits of this interesting little fish to remark upon its behavior during the rest of the year.

[3] B. W. Evermann and H. W. Clark, "Lake Maxinkuckee," Dept. of Conservation, State of Indiana, 1920.

[4] A. C. Johansen, "On the Migrations of the Herring." Concluding remarks, p. 27, in *Journal du Conseil* (Perm. Internat. Expl. de la Mer), Vol. II, No. 1, Copenhagen, 1927.

manifest itself with particular strength shortly after the breeding season ended, it must, on the other hand, also be remembered that this period of assumed scattering would also be coincident with the period of the probably greatest reduction of all manifestations of sexual attraction, which may otherwise lend their support to the maintenance of a schooling behavior, in the absence of externally perceptible sex differentiations in this species.

It might seem from these considerations as though indiscriminate schooling behavior and differential sex orientation could be perfectly equivalent responses of the identical psychological mechanism to the varying conditions of sexual di- or mono-morphism. Certain observations, however, while substantially confirming the relationship between schooling behavior and sex attraction as above made out, at the same time clearly tend to show that there must also be an independent complex for each of the two phenomena. It would, as already indicated in the above-discussed example of the herring, be quite unnatural and entirely discordant with all our knowledge about such matters to assume that the mechanism of sexual attraction should be equally well developed at all stages in the life of the individual fish, or that it should always be equally active after the mature stage has been reached. We nevertheless find the schooling performance much more frequent among young fishes than among their adults, as already above mentioned. We further observe how, for instance, the Cyprinodont, *Fundulus diaphanus,* as described by Evermann and Clark (see above), in spite of permanent and conspicuous sex dimorphism, is yet habitually living in large schools, which, however, show a distinct tendency toward partial or complete disintegration into smaller schools or single pairs during the spawning season, when the sexual instinct becomes active. On the basis of such observations of increased schooling during periods of presumably undeveloped or less active sexual instincts it therefore seems logical to assume that the schooling instinct as

such must be a separate element of a probably very wide distribution in the nervous organization of fishes, while the occurrence of its external manifestations in the form of actual schooling behavior is largely dependent upon and explainable by the promoting or suppressing influence from the sexual orientation of the individual, according to the varying degrees of externally perceptible sex differentiation.

It must, however, not be overlooked that numerous other factors also must be expected to exert a great, if not so general, influence upon the schooling performances of the individual species. The fact that a pelagic habitat seems more conducive to schooling than does a benthonic mode of life has already previously been mentioned by the writer, and the survival value of social or solitary tendencies according to the entire habit-pattern of the various forms, their feeding, locomotion, etc., is a factor of obvious importance in the single cases.

THE LONGEVITY OF ENCYSTED COLPODAS

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IN 1929 Dawson and Mitchell reported the recovery of a number of motile organisms from Cuban hay which was gathered by the senior author on May 1, 1925. These included a variety of micro-organisms, *e.g., Colpoda cucullus, Oxytricha* sp., *Spathidium spathula, Podophrya fixa, Physarum cinereum* and nematodes of the genus Cephalobus. Cultures made in May, 1929, yielded only the following: Colpoda, Oxytricha, Spathidium, Physarum and Cephalobus. In this earlier paper the literature concerning the longevity of infusorian cysts was reviewed and need not be referred to here.

A series of cultures was made beginning August 21, 1930, to ascertain which of these organisms could still be recovered from the encysted state after a period of approximately five years and four months. Infusions of the dry hay were made using glass distilled water and sterile Tashmoo spring water. For the cultures with the larger quantities of medium, finger-bowls were used; for the smaller quantities, Syracuse watch-glasses. The experiments were conducted at room temperature which, during the period, varied from 22° to 30° C. In all but one of the infusions made living motile organisms were recovered. All cultures were examined at regular short intervals after they were begun (with the exception of one or two of the Syracuse watch-glass cultures) for the purpose of recording the first appearance of the excysted organisms. The record of the different infusions made is summarized as follows:

Controls were carried in each series. In none of these were any organisms seen. A study of the record of the various series shows that motile colpodas were recovered from each of the four types of infusions and active nema-

SERIES I

Number	Date	Medium	Amount of medium	Weight of hay	First observation of animals (in hours after starting culture)	Number seen at this time	Name	Remarks
I	Aug. 21	Glass distilled water	100 cc	1.9 g	24½	6	*Colpoda cucullus*	Number of colpodas increased up to about 8 days and then slowly declined.
					41	14	* *Cephalobus elongatus*, *Aphelenchus* sp., *Rhabditis* sp.	
II	" "	"	"	.9 g	24½	3	*Colpoda cucullus*	Similar to I except the number of colpodas was slightly less at any time.
					41	10	Nematodes as above	
					264		*Ameba* sp.	The amebae observed were a small species resembling *A. bigemma* and were present at this time in considerable numbers.
III	Sept. 2	"	"	1.5 g	18½	5	*Colpoda cucullus*	Number of colpodas reached a maximum after two days, about 50 animals counted in a 4 mm field using binocular dissecting microscope. Slight decline after four days.
					120	few	Nematodes as in I	
IV	" "	"	9 cc	.13 g	.....	.....	.....	
V	" "	"	9 cc	.07 g	44	many	*Colpoda cucullus*	Maximum numbers reached on Sept. 4. Culture very gradually declining when discontinued on Sept. 9.

* Identifications made by Dr. Gerald Thorne, nematologist, U. S. Department of Agriculture.

SERIES II

Number	Date	Medium	Amount of medium	Weight of hay	First observation of animals (in hours after starting culture)	Number seen at this time	Name	Remarks
I	Sept. 2	Hay infusion (.44 g timothy hay in glass distilled water—only clear fluid used)	100 cc	1.5 g	18¾	1	*Colpoda cucullus*	Later history as in number III, Series I.
II	" "	Hay infusion. Same composition as above	9 cc	.13 g	23½	2	" "	Later history as in number V, Series I.
III	" "	"	"	.07 g	23½	1	" "	"

SERIES III

I	Aug. 23	Tashmoo spring water	100 cc	.9 g	22¾	1	*Colpoda cucullus*	By Aug. 25 culture was flourishing and continued with numerous colpodas until Aug. 27, when numbers decreased strikingly until almost no colpodas were present.
					23¼	1	Nematode as in Series I	
II	Sept. 2	"	"	1.5 g	22	2	*Colpoda cucullus*	Maximum numbers reached Sept. 5 and declined rapidly thereafter.
					64	1	Nematode as above	
III	" "	"	9 cc	.13 g	23¾	1	*Colpoda cucullus*	Later history as in number II of this series.
IV	" "	"	"	.07 g	44	many	" "	"

SERIES IV

Number	Date	Medium	Amount of medium	Weight of hay	First observation of animals (in hours after starting culture)	Number seen at this time	Name	Remarks
I	Sept. 2	Hay infusion in Tashmoo spring water. (Same concentration as in Series II)	100 cc	1.5 g	$20\frac{1}{4}$	1	*Colpoda cucullus* Nematode as in Series I	Reached maximum concentration on Sept. 4; numbers declined after Sept. 5.
					64	1		
II	" "	"	9 cc	.13 g	$23\frac{3}{4}$	3	*Colpoda cucullus*	Numbers small at all times.
III	" "	"	"	.07 g	$23\frac{3}{4}$	1	" "	Numbers slightly greater than in No. II of this series, declining after Sept. 8.

todes from all but those of Series II. It should be mentioned that in addition to the organisms listed in the various series small flagellates which have not been identified occurred generally. It is interesting to note that at this period certain of the organisms, *i.e., Oxytricha,* Spathidium and Physarum, which were recovered in 1929, did not appear in any of the series. It is possible that these organisms may still be alive in the encysted condition. This seems highly improbable, however, since representative samples of the hay were used in every large culture. The distribution of the colpoda cysts generally throughout the hay is proved by the fact that from the very small samples used in the Syracuse watch-glass cultures colpodas were recovered in all cases except one (Culture IV, Series I). In this culture it is significant that no leaves of hay were present. It is believed that the round hay stalks used here did not afford an adequate surface on which the colpoda protection cysts could form.

The experiments were planned to test the relative merit of different kinds of media in inducing excystment and multiplication in the original medium after excystment. The records show that while organisms were recovered in motile condition in each type of medium, multiplication continued longer and a higher concentration of colpodas was reached in glass distilled water than in the media made from spring water. It is noteworthy, however, that the nematodes of the various genera identified multiplied only in the spring-water cultures. This multiplication went on even after the decline of the colpodas in these cultures.

In the rapidly multiplying colpoda cultures a great discrepancy in size was noted as in the previous paper, Dawson and Mitchell (1929). Small individuals measuring from 25 to 31 μ in length were isolated in sterile culture medium, and upon multiplication gave rise to individuals up to 50 μ in length. On the other hand, large individuals measuring from 55 to 60 μ in length were isolated from the original cultures and gave rise to

progeny of various lengths from 25 to 60 μ. Both large and small colpodas are identical in external morphological details. It is therefore certain that we are here concerned with but a single species of *Colpoda.* This discrepancy in size commonly occurs in rapidly dividing protozoan cultures. It was noted that the division cysts varied greatly in size while the cultures were flourishing, the usual number of organisms coming from each cyst being either two or four. In one case, however, a division cyst was observed from which eight colpodas of the smallest size (25 μ long) emerged.

This account constitutes a record of the greatest longevity of the protection cysts of *Colpoda cucullus* hitherto reported.

SHORTER ARTICLES AND DISCUSSION

THE INFLUENCE OF MANGANESE IODIDE AND ETHYL BUTYRATE ON RATS FURNISHED A VITAMIN A–FREE DIET

PREVIOUS studies from this laboratory having indicated that ferrous iodide exerts beneficial effects when furnished in minute quantities to rats on a Vitamin A deficient diet (Chidester, Eaton and Thompson, 1928), we decided to test manganese iodide also.

Thirty-seven young rats, about 30 days of age and averaging 35 gm in weight, were placed on a diet deficient in Vitamin A and low in Vitamin D (Sherman No. 380) on October 14, 1929, and when greatly depleted to the point of xerophthalmia and weight loss, were, on January 25, separated into lots with split litters and corresponding weights and isolated in individual round cages. With the exception of the controls, they were then furnished with 1/100 mgm of irradiated ergosterol daily from January 25 to February 18, when Lot 1 received 5 drops of MnI_2 and Lot 2 received 3 drops of MnI_2 in a solution such that the first lot received .0005 grain of I_2 and the second lot received .0003 grain of I_2, as in an experiment with FeI_2 elsewhere recorded.

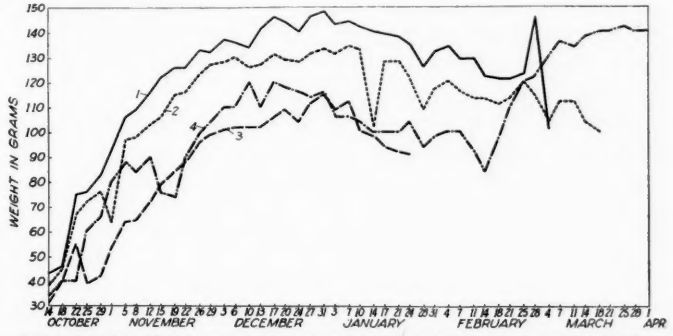

FIG. 1 MANGANESE IODIDE AND ETHYL BUTYRATE WITH ERGOSTEROL

Lots and Treatment

1. Sherman No. 380, ergosterol, January 25; 5 drops MnI_2, February 18; 5 drops ethyl butyrate, February 22.
2. Sherman No. 380, ergosterol, 3 drops MnI_2, 5 drops ethyl butyrate.
3. Sherman No. 380, Control.
4. Sherman No. 380, Cod Liver Oil, control.

RESULTS

Lot 1 continued to utilize stored foods and to increase in weight until December 31, then rapidly fell in weight and showed signs of deficiency in A and D until they were furnished ergosterol on January 25. From that date until February 6 the effect of the added Vitamin D was quite evident, but it rapidly wore away until on February 17 the average weight had gone down to 121 gm, the weight shown on November 14 before stored foods had been depleted. After the addition of MnI_2 on February 17, marked stimulation of growth occurred, the curve rising noticeably. Again, the average increased rapidly with the addition of 5 drops of ethyl butyrate on February 22. The death of all but three of the lot occurred by March 4, however. It would seem that manganese iodide is not so satisfactory a catalyzer as ferrous iodide.

Lot 2 became depleted rather rapidly from about January 10 to January 25, the date when they began receiving 1/100 mgm of irradiated ergosterol. The stimulating effect of this Vitamin D addition lasted until February 5, then the curve began to fall until a low point was reached on February 16. On February 18 the administration of 3 drops of MnI_2 (.0003 grain of iodin) daily raised the average 9 gm in 4 days. The addition of 5 drops of ethyl butyrate on February 22 failed to improve the condition of the lot, and on March 4 only 2 survived in a much depleted condition, although exhibiting no signs of xerophthalmia.

Lot 3, controls on Sherman No. 380 diet, were depleted in about 9 weeks.

Lot 4, a control lot, received ergosterol on January 25, and showed some slight effects, but began to grow rapidly when the ergosterol was replaced by one drop daily of cod-liver oil beginning February 14.

CONCLUSION

We feel that in this experiment we have possibly demonstrated a slight benefit from ethyl butyrate, added to a diet containing MnI_2, but that we must seek other fats. Theoretically, we should be able to utilize highly unsaturated fatty acids which would balance the iodin if it should at any time exceed its usefulness as a catalyzer in vitamin-deficient rats.

Thirty-one young rats, about 30 days of age, averaging about 35 gm in weight, were selected for an experiment in which

minute quantities of MnI_2 were added to irradiate ergosterol and furnished animals that had been depleted for more than 8 weeks on a diet (Sherman No. 380) deficient in Vitamin A and low in Vitamin D.

The iodin content of the solution was such as had been proved beneficial in combination with iron, and elsewhere described, and was such that 5 drops of MnI_2 contained .0005 grain of iodin, and 3 drops contained .0003 grain of iodin. The solutions were delivered from pipettes graduated so that each drop represented 1/15 of a cc.

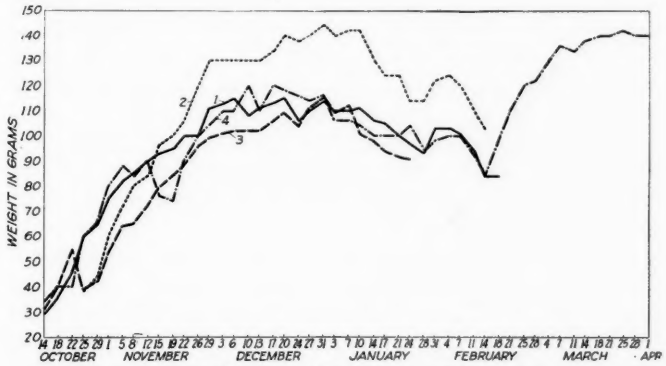

FIG. II MANGANESE IODIDE. WITH ERGOSTEROL

Lots and Treatment

1. Sherman No. 380; ergosterol, January 25; 5 drops MnI_2, February 18.
2. Sherman No. 380; ergosterol, 3 drops MnI_2.
3. Sherman No. 380; Control.
4. Sherman No. 380; ergosterol, January 25, replaced by Cod-liver Oil, February 14.

Results

Both Lots 1 and 2 showed only slight stimulation of growth after the addition of ergosterol, and apparently could not tolerate the added MnI_2. It is possible of course that we have underestimated the value of the ethyl butyrate added in Experiment 1 (Graph 1). Our general reaction, however, is that we must seek fats or fatty acids that will be less readily volatilized and that, although they are Vitamin A free, will aid in restoring our fat-iodin balance.

General Conclusion

These experiments, together with other preliminary studies performed with a small number of animals, would seem to indi-

cate that MnI_2, given in the presence of irradiated ergosterol, does not prolong the lives of rats on an A-deficient diet more than six weeks and adds little to their growth, even when small quantities of ethyl butyrate are added.

In comparison with these results we have elsewhere (Chidester, Eaton and Thompson, 1930) discussed experiments in which ferrous iodide, with the same daily dosage of iodin as we have used in the present experiment, not only cured and protected rats from xerophthalmia, but induced growth and preserved the animals for more than fourteen weeks after profound depletion.

Studies of McCarrison (1927) have shown that MnO_2 in doses of .880 mgm per day caused retardation of growth in rats on a normal diet, the signs becoming evident at the end of 32 days. But $MnCl_2$ in daily doses of 0.0327 mgm for 53 days caused accelerated growth, particularly in the male rats.

Aso (1902) has shown that manganese sulphate in solution (0.002 per cent.) stimulates growth in radishes, peas and wheat plants, but that this effect is counteracted by the addition of iron to the solution.

Bishop (1928) showed that, without manganese, iron assimilation was normal in plants, but that growth ceased after 5 to 7 weeks. Calcium counteracted the toxicity of high manganese concentrations. It may be conjectured that in our experiments irradiated ergosterol made available a greater supply of calcium which reduced the toxic effects of the manganese. In McCarrison's studies it is also conceivable that the $MnCl_2$ brought in sufficient chlorine to act as an oxidant on the ferrous salts contained in the organs of his experimental rats.

While ethyl butyrate may prove beneficial in some deficiencies, it is probable that other fats or fatty acids will prove more palatable and perhaps more suitable, as slight increases in iodin dosage prove quite injurious to depleted animals. Again, we must consider the possible beneficial rôle of unsaturated fatty acids (Burr and Burr, 1930) presented with iodin (Chidester, 1930) under such profound nutritional deficiencies as we have experimentally produced.

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THE *CREPIS SETOSA* CHROMOSOMES PRESENT IN *CREPIS ARTIFICIALIS;* A CORRECTION OF TISCHLER'S STATEMENT CONCERNING AUTOSYNDESIS AMONG *SETOSA* CHROMOSOMES

In his paper upon the correlation of cytology and taxonomy in plants Tischler (1929) refers to the origin of the chromosomes of *Crepis artificialis,* a description of which was published by the present writers. Tischler has obviously misunderstood the original description and consequently credited *Crepis artificialis* with a chromosome origin not found by the authors, who wish to take this opportunity of calling attention to the correct derivation of the chromosomes of this new species which was detailed in their paper (Collins, Hollingshead and Avery, 1929).

In the following quotation from Tischler's paper it is stated that the four chromosomes (haploid number) from *Crepis setosa* conjugated to form two pairs during meiosis in the species hybrid, *Crepis biennis* $(n-20) \times C.$ *setosa* $(n-4)$.

> Ganz eindeutig scheint mir aber für eine Bindung nicht homologer Chromosomen der Fall zu sprechen, den Collins, Hollingshead u. Avery (1929) beschreiben. Es handelt sich um den sogenannten *Crepis artificialis.* Er ist hervorgegangen aus der Kreuzung *Crepis biennis* (20) × *Cr. setosa* (4). Nicht nur die 20 Chromosomen der ersten Art schlossen sich autosyndetisch zu Paaren zusammen, *sondern auch die 4 der zweiten.*[1] Wir erhalten so 12 Gemini. Und es ist doch die Zerlegung der vier chromosomen in zwei einander "homologe" Paare mehr also unwahrscheinlich (page 47–8).

The following excerpts from our paper are given here to show that the statement regarding autosyndesis of the four *Crepis setosa* chromosomes was not warranted.

[1] Italics inserted by present authors.

In *setosa* the four chromosomes are easily distinguishable, figure 1a. One long chromosome has a subterminal constriction to which a large satellite is attached by a thin thread. A second, about equally long, has the subterminal constriction but lacks the satellite. The third is similar to the second but is shorter, while the fourth is very small with a subterminal constriction.

The two *setosa* chromosomes present in the *artificialis* complex are quite readily recognized by their morphological characters and each is present twice. These are the first and fourth types mentioned above . . . (p. 309).

In the F_1 resulting from crossing *artificialis* with *setosa*, plants containing 10 chromosomes from *biennis* and 6 from *setosa* were secured. The latter included the two pairs present in *artificialis* plus one member of each of the two pairs not represented in *artificialis* . . . (p. 316).

The *setosa* chromosomes in *artificialis* can be identified as the long chromosome pair with a large satellite and the smallest pair of *setosa* . . . (p. 319).

It was also pointed out in the paper referred to by Tischler and likewise in a previous publication (Collins and Mann, 1923) that in interspecific crosses involving *C. biennis* the haploid *biennis* chromosomes (20) conjugated to form 10 pairs but that the chromosomes of the other species remained as univalents and were distributed at random during meiosis.

It is hoped by this note to correct the statement that autosyndesis took place in the haploid sets of both species which contributed to the origin of *Crepis artificialis*.

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